Imaris V 7.3.1
Reference Manual

BITPLANE
Scientific Software

1 Preface

This Reference Manual provides a description of all menu entries, display modes, functions and parameters.

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Imaris Reference Manual V 7.3.1

1.1 Getting Familiar

Today, all optical microscopes commercially available can record several channels simultaneously to produce multi-channel images. Imaris is an application designed to visualize such microscopic data. Imaris uses a special file format to store images with parameters and can incorporate image files for all major microscopes and image acquisition systems. The images can be viewed in several different ways and processed to provide the optimum amount of information from 2D or 3D still images, time series, and animations.

Once a data set has been loaded into Imaris, individual parameters such as channel colors, geometrical settings or voxel sizes can be adjusted. Imaris has a variety of tools available, such as cropping, threshold cutting and filters for processing the images to bring out the required details.

It provides 8 different viewing functions for the visualization and production of high quality images for presentation and storage:

- A Slice viewer.
- A Section viewer for simultaneous viewing along three coordinate axes.
- A Gallery viewer for slice image overview and selection.
- The Easy 3D viewer provides a quick image view.
- The Surpass viewer, which offers numerous tools for data preparation, presentation and manipulation of different types of data display as well as any combination of them and the ability to define, combine and group an arbitrary number of objects out of a set of viewing objects.
- Animations can be created from the Slice and 3D modes, or with the key frame animator in Surpass.
- InMotion is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression by a smooth animation of the view.
- A viewer for Colocalization computation.

It is easy to navigate within the Imaris modules because the frequently used toolbars, menus, and interactive controls remain the same, and can all be operated with the mouse buttons.

1.2 Getting Started

The software is delivered on a standard CD or downloaded from www.bitplane.com. The CD includes a folder containing the necessary manuals, or the manuals can be downloaded.

Minimum hardware/software requirements are:

- Windows NT 4.0, or a more recent version, Windows 2000, XP, Vista, OS X 10.5.0 (Leopard) or higher
- CD-ROM
- Graphics card with 3D accelerator
- Network facilities for image import from the microscope
- 512 MB RAM (> 1 GB recommended)

Bitplane also recommends:

- A database for storing images (e.g., Image Access)

Installation

To install the software, please proceed as follows:

- Insert your Imaris CD-Rom in the computer.
- Follow the instructions on the screen.
- The installation is completed automatically.

Licensing

To run the Imaris system, the appropriate licenses for the required modules, such as the Imaris base (including Surpass), ImarisTime, ImarisColoc or Imaris MeasurementPro. Without licenses, the Imaris can only be run in a restricted mode. In case of any license problems, please refer to the support information on our website www.bitplane.com for detailed instructions.

Starting Imaris

Imaris can be started by one of the following methods:

- Double-click on the Imaris icon (we recommend copying the icon to the desktop).
- Drag the icon of an image or a file to the Imaris program icon.
- Imaris can be started directly from the Image Access database.

The software opens with the main screen.

Supported File Formats

Imaris (as of version 6.0.0) can read the following file formats, i.e. it can read the image and the parameters.

- Andor: Multi-TIFF series (*.tif, *.tiff)
- Andor: MM (*.kinetic)
- Andor: iQimage (*.kinetic)
- Applied Precision, Inc: DeltaVision (*.r3d, *.dv)
- Biorad: MRC-600, MRC-1024 (series)(*.pic)
- BioVision: IPLab Mac (*.ipm)
- Bitplane: Imaris 5.5 (*.ims)
- Bitplane: Imaris 3.0 (*.ims)
- Bitplane: Imaris 2.7 Classic/Old (*.ims)
- Bitplane: Imaris Scene File (*.imx)
- BMP (adjustable file series) (*.mrc, *.st, *.rec)
- Carl Zeiss: LSM 510 (*.lsm)
- Carl Zeiss: LSM 410, LSM 310 (*.tif, *.tiff)
- Carl Zeiss: Axiovision (*.zvi)
- Compix:Simple PCI (*.cdx)
- Image Cytometry Standard: ICS - used by Nikon, Huygens, and others (*.ics, *.ids)
- Gatan Digital Micrograph (serie):(*.dm3)
- Leica: TCS-NT (*.tif, *.tiff)
- Leica: LCS (*.lei, *.raw, *.tif, *.tiff)
- Leica: series (*.inf, *.info, *.tif, *.tiff)
- Leica: Image File Format (*.lif)
- MetaSystem: Meta Viewer (*.imv)
- Molecular Devices: Metamorph STK (series) (*.stk)
- Molecular Devices: Metamorph ND (*.nd)
- MRC: primarily electron density volumes as in cryo-EM (*.mrc, *.st, *.rec)
- Nikon: ND2 (*.nd2)
- Olympus: FluoViewTIFF (*.tif, *.tiff)
- Olympus: 1000 OIF (*.oif)
- Olympus: 1000 OIB (*.oib)
- Olympus: Cell^R 1.1/standard (*.tif, *.tiff)
- Olympus:Virtual Slide Image (*.vsi)
- Opellab: liff (series) (*.liff)
- Openlab: raw (*.raw)
- Open Microscopy Environment XML (*.ome)
- Open Microscopy Environment TIF (*.tif, *.tiff)
- Perkin Elmer: UltraView (*.tim, *.zpo)
- Prairie Technologies: Prairie (*.xml, *.cfg, *.tif, *.tiff)
- Scanalytics: IPLab (*.ipl)
- Slide Book (*.sld)
- TIFF (adjustable file series) (*.tif, *.tiff)
- TILL Photonics: TILLvisION (*.rbinf)

Plus it can read general TIFF series (or BMP series) of the format aaaNNN.tif (where a is a character and N is a number).

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2 Menu File

Open...	Ctrl + O
Revert to File	Ctrl + R
Save	
Save as...	Ctrl + S
Batch Convert...	
Scene Viewer...	
Batch	
Import Scene...	Ctrl + L
Export Scene...	Ctrl + E
Snapshot...	Ctrl + T
Exit	Ctrl + Q

See also:
Addendum - Mouse & Keyboard Mac

2.1 Open

Data sets can be loaded from various file formats.

Image File Series

If the data set consists of a whole series of images, each stored as individual file, select only one file to open and the system will automatically load the rest of the images that belong to the data set.

- In the menu select File – Open.
- Select file type from the Files of Type pull-down menu.
- Select a file name from the list and click **Open** or double-click on the requested file entry.

The file is loaded.

iQ files in Imaris

To open iQ files in Imaris locate and select the **DisckInfo5.kinetic file**.
Clicking on the '**Settings**' button opens a new window with a list of images and image information.
Check the box Thumbnail Preview to activate an image preview.
Select the required image and click on the **OK** button.
Than click again on the **Open** button. This will instantly create 3D volume rendered image of the selected data set.

File Formats

Imaris can read the following file formats, i.e. it can read the image and the parameters.

- Andor: Multi-TIFF series (*.tif, *.tiff)
- Andor: MM (*.kinetic)
- Andor: iQimage (*.kinetic)
- Applied Precision, Inc: DeltaVision (*.r3d, *.dv)
- Biorad: MRC-600, MRC-1024 (series)(*.pic)

- BioVision: IPLab Mac (*.ipm)
- Bitplane: Imaris 5.5 (*.ims)
- Bitplane: Imaris 3.0 (*.ims)
- Bitplane: Imaris 2.7 Classic/Old (*.ims)
- Bitplane: Imaris Scene File (*.imx)
- BMP (adjustable file series) (*.mrc, *.st, *.rec)
- Carl Zeiss: LSM 510 (*.lsm)
- Carl Zeiss: LSM 410, LSM 310 (*.tif, *.tiff)
- Carl Zeiss: Axiovision (*.zvi)
- Compix:Simple PCI (*.cdx)
- Image Cytometry Standard: ICS - used by Nikon, Huygens, and others (*.ics, *.ids)
- Gatan Digital Micrograph (serie):(*.dm3)
- Leica: TCS-NT (*.tif, *.tiff)
- Leica: LCS (*.lei, *.raw, *.tif, *.tiff)
- Leica: series (*.inf, *.info, *.tif, *.tiff)
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- Olympus: FluoViewTIFF (*.tif, *.tiff)
- Olympus: 1000 OIF (*.oif)
- Olympus: 1000 OIB (*.oib)
- Olympus: Cell^R 1.1/standard (*.tif, *.tiff)
- Olympus:Virtual Slide Image (*.vsi)
- Opellab: liff (series) (*.liff)
- Openlab: raw (*.raw)
- Open Microscopy Environment XML (*.ome)
- Open Microscopy Environment TIF (*.tif, *.tiff)
- Perkin Elmer: UltraView (*.tim, *.zpo)
- Prairie Technologies: Prairie (*.xml, *.cfg, *.tif, *.tiff)
- Scanalytics: IPLab (*.ipl)
- Slide Book (*.sld)
- TIFF (adjustable file series) (*.tif, *.tiff)
- TILL Photonics: TILLvisION (*.rbinf)

Add to Batch Button
Add to Batch button places the command into Batch coordinator.

See also:
Menu File - Batch Convert ...

2.1.1 Reader Configuration

Read only one Time Point

Loads a single time point of a time series.

Resampling Open...

The Resampling Open dialog box can be used as preview before loading a data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This can be particularly important when reading large data sets over a network.

The revision applies to all views in Imaris and in Surpass.

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- Select menu File - Open or click on the button **Open** in the Main toolbar.
- Click on a file to highlight it and click on the button **Resampling File Open ...** .

Image Preview
The view on the right side displays as image preview a single time point of the data set. Right-click in the view and move the mouse pointer to the right to increase brightness. Move the pointer upwards to increase contrast.

Original Size
Display of the original file size.

Subsampling Factor

X,Y,Z, Ch, T
You have the option to specify the Subsampling Factor, i.e., the fraction of data points to be retained. The subsampling factor can be specified for the x-, y-, z-directions, the channels (Ch), and the time points (T).

Crop Limits (Min/Max)

X, Y, Z, Ch, T, From, To
The Crop Limits (Min/Max) for the x-, y-, z-directions, the channels (Ch), and the time points (T) can also be specified.

Resampled Size
Display of the resampled file size.

- Click on **OK** and the data set is cropped and resampled while loading.

See also:
Menu Edit - Image Properties... - Geometry
Menu Edit - Crop Time...
Menu Edit - Resample Time...
Menu Edit - Crop 3D...
Menu Edit - Resample 3D...

2.1.2 Settings

In the Settings you specify options for reading certain file formats.

Leica LCS Settings

A LeicaLCS data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

- Select File - Open or click **Open** in the Main toolbar.
- Select Files of Type: Leica LCS.
- Click on the *.lei file to highlight it and click Settings.

A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).
By checking the box **Thumbnail Preview** possibility of activating an image preview is available**.** The individual images that belong to that stack will display on the left side.

Select the required image stack on the left side of the dialog box.
- Click **OK** to open the image.

Leica LIF Settings

A LeicaLIF data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

- Select File - Open or click **Open** in the Main toolbar.
- Select Files of Type: Leica LIF.
- Click on the *.lif file to highlight it and click Settings.

A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).
By checking the box **Thumbnail Preview** the possibility of activating an image preview is available**.** The individual images that belong to that stack will display on the left side.

Select the required image stack on the left side of the dialog box.
- Click **OK** to open the image.

Adjustable Tiff Series Reader Settings

If the data set consists of a series of images, individual images can be sorted according to various dimensions (i.e., slices, channels, time points, dimension sequence). The selected sequence is shown in the File Arrangement panel. The reader can handle tiff series with single and multiple running numbers.

- Select the menu File - Open or click **Open** in the Main toolbar.
- Select Files of Type: Tiff (adjustable file series) and not Tiff (series) from the drop-down list.
- Open the folder containing the series.
- Click Settings, which is grayed out if the file type selector is on automatic or if the current directory does not contain a series.
- Use Apply Automatic File Filter, Apply, Dimensions, Dimension Sequence, described as follows, to define the series.

Apply Automatic File

This is activated automatically when the dialog is opened. It has the same logics as the classic TIFF series reader of Imaris and will pre-select the first series detected in the directory. Be aware that you may not see all files in the directory.

Button **Apply**
Press this button to use the regular expression to the left and select all files in the current directory that match the criterion, i.e.

- *.tif selects all files with the ending *.tif.
- myfile*.tif selects all files that start with "myfile" and are followed by any letter or digit and by the extension *.tif.
- myfile??.tif selects all files that start with "myfile" and are followed by two letters or digits and by the extension *.tif.

Please note: Depending on the filter, not all files in the directory may be visible. Selecting the required file may take some time because every file is opened but only files with identical xy-dimension are chosen.

Dimensions
- Define the dimensions of the image starting with Slices (Z), Channels (Ch), and Time Points (T).

Please note: The total number of files in the series, as defined by your selection criteria, displays below the Time Points input box. Selecting the required file may take some time because every file is opened but only files with identical xy-dimension are chosen.

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Dimension Sequence

- Defines how the individual images, which are sorted alphabetically, are to be assigned to Slices (Z), Channels (Ch), and Time Points (T).
- Click **OK** to return to the Open dialog window.
- Click **Resampling Open** to open the Resampling dialog box or click **Open** to open the image.

2.2 Open in new Window...

This command allows you to open multiple Imaris files.

2.3 Open iQ File ...

Clicking on the **Open iQ files** opens the **Image Selection** window with a list of images and image information.

Check the box Thumbnail Preview to activate an image preview.
Select the required image and click on the OK button.
This will instantly create a 3D volume rendered image of the selected data set.

Search File Name
Search for all files that meet a desired criteria

Resample Open Button
Resampling Open...

The Resampling Open dialog box can be used as preview before loading a data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data.

Image Preview
The view on the right side displays as image preview a single time point of the data set. Right-click in the view and move the mouse pointer to the right to increase brightness. Move the pointer upwards to increase contrast.

Original Size
Display of the original file size.

Subsampling Factor

X, Y, Z, Ch, T
You have the option to specify the Subsampling Factor, i.e., the fraction of data points to be retained. The subsampling factor can be specified for the x-, y-, z-directions, the channels (Ch), and the time points (T).

Crop Limits (Min/Max)

X, Y, Z, Ch, T, From, To
The Crop Limits (Min/Max) for the x-, y-, z-directions, the channels (Ch), and the time points (T) can also be specified.

Resampled Size
Display of the resampled file size.

- Click on **OK** and the data set is cropped and resampled while loading.

2.4 Revert to File

Re-opens the actual data set.

2.5 Save

To save an existing dataset click **Save** in the File menu or Save icon on the Main toolbar.

2.6 Save as ...

Saving in Imaris format is recommended whenever the data set is cropped or the parameters changed. Saving a data set in Imaris file format provides the advantage of a faster loading process and the possibility of using thumbnails. In addition, parameters are saved with the images.
The standard way to save your data and Imaris scene is simply to choose **Save as** from the File menu and give an appropriate name. The file extension, suggested as the default, is *.ims. The single created Imaris file contains both the image and the scene data.
You can save the file when you are done or if you want you can stop in the middle and resume work later. Simply, choose **Save as** from the File menu and give the file a name you will remember. If you then close the file and reopen it at a later time, you can resume work from where you left off.

- In the menu bar select File - **Save as...**. The Save as window displays.
- Select the directory and enter the name for the file to be saved or confirm the suggestion.
- Select the requested file format and click **OK**.

The data set is saved.

Available File Formats in Imaris

Bitplane: Imaris 5.5 (*.ims)
Bitplane: Imaris 3.0 (*.ims)
Bitplane: Imaris 2.7 (Classic)(*.ims)
MetaSystems: MetaViewer (*.imv)
Tiff (series)(*.tif *.tiff)
RGBA-Tiff (series)(*.tif *.tiff)
ICS file (*.ics *.ids)
Olympus: cell^R 1.1/standard (*.tif *.tiff)
Open Microscopy Environment XML (*.ome)
Open Microscopy Environment Tiff (*.tif *.tiff)
BMP (series)(*.bmp)
Move file (slice animation) (*.avi, *.mov, *.tif, *.tiff)

Export and Import Scene File

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Surpass Scene and can be stored in a Scene file with the extension *.imx. The Surpass Scene can be loaded again to the same data set or to another data set. For details please refer to chapter Surpass View - Overview - Scene File Concept.

Button **Add to Batch**
Add to Batch button places the Save command into Batch coordinator. The **Save** command can generate a variety of different output files.
The type of output files is defined in the **Writer** parameter in the Value column. The parameter **Writer** value is automatically set to be in accordance with the chosen option for file type in the **Save as** window in Imaris.

File type chosen in the Save as window	Writer value - output file type

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Bitplane: Imaris 5.5 (*.ims)	Imaris5
Bitplane: Imaris 3.0 (*.ims)	Imaris3
Bitplane: Imaris 2.7 (Classic)(*.ims)	Imaeris2
MetaSystems: MetaViewer (*.imv)	MetaViewer
Tiff (series)(*.tif *.tiff)	SeriesAdjustable
RGBA-Tiff (series)(*.tif *.tiff)	TiffSeriesRGBA
ICS file (*.ics *.ids)	ICS
Olympus: cell^R 1.1/standard (*.tif *.tiff)	OlympusCellR
Open Microscopy Environment XML (*.ome)	OmeXml
Open Microscopy Environment Tiff (*.tif *.tiff)	OmeTiff
BMP (series)(*.bmp)	BMPSeries
Move file (slice animation) (*.avi, *.mov, *.tif, *.tiff)	MovieFromSlices

See also:
Menu File - Batch Convert...

2.6.1 Advanced Save Options

Button **Format Settings...**
A click on the button **Format Settings...** opens the Imaris Save Options window.

Window: Save Options

Bitplane: Imaris 5.5 (*.ims)

Enable Compression
Check the box to use compression.

Export Scene
Check the box to save the Imaris scene.

Bitplane: Imaris 3.0 (*.ims)

Time Series
Save as Single File
The time series are saved in a single file.

Save as Multiple Files
For each time point a new file is generated.

Compression
LZW Compression
Check the box to use an LZW compression.

Add to ImageAccess Database

Check the box to add the file to the ImageAccess database.

2.7 Batch Convert ...

With the Imaris File Converter you can convert various image file formats to the Imaris file format *.ims. Select the menu entry Batch Convert... and the Imaris File Converter window displays. From the Windows Start menu select "ImarisFileConverter".

Input

Drag & Drop Files, or click the Button below to add Files.

Button **Add Files ...**
The window Select Files for Conversion displays. Choose the respective file and click on the **Add Files...** button.

Thumbnail

Here you can select the appearance of the thumbnail in Imaris.

Middle Slice
Thumbnail is the middle slice.

MIP
Thumbnail in the display mode Maximum Intensity Projection. A Maximum Intensity Projection is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

Blend
Thumbnail in blend projection. Blends all values along the viewing direction and includes their transparency.

Output

Same Folder as Input File
You find the converted image(s) in the same folder as the input files.

Special Folder
Here you can select another folder for the converted image(s). Either type in the respective path or use the button **Browse**.

Button **Browse**
Click on this button to browse for the special folder.

Button **Set for All**
Click on this button and all converted image(s) are saved in the designated folder.

Format
Here you can select the format of the output files. The standard formats are:
Bitplane: Imaris 5.5 (*.ims)
Bitplane: Imaris 3.0 (*.ims)
Bitplane: Imaris 2.7 (Classic)(*.ims)

To add additional formats please refer to chapter Menu File - Batch Convert ... - Preferences.

Input

Here you find the selected input file path(s).

Click on the input path to open the window Series Reading Sequences to adjust additional parameters for the conversion. Please refer to chapter Menu File - Open - Settings for details.

Output

Here you find the selected output file path(s).

Click on the output path to open the window Imaris Save Options to adjust additional parameters for the conversion. Please refer to chapter Menu File - Save as ... - Advanced Save Options for details.

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Clear Row

To clear a row click on the red cross at the end of the row.

Button **Clear**
Click on this button to clear all rows in the table.

Button **Start**
Click on this button to start the conversion.

See also:
Menu File - Open - Reader Configuration
Menu File - Open - Settings
Menu File - Save as ... - Advanced Save Options
Menu File - Batch Convert ... - Preferences

2.7.1 Preferences

Button **Preferences**
Click on this button to open the Preferences window.

Window: Preferences

Data Cache

Imaris utilizes advanced data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.
Cache File Paths displays the cache file location.

Please note: Setting the Cache File location to an unused disc, improves the performance when working on larger images. This benefits a lot from a fast SSD with a minimum size of 10 times the biggest dataset, which will be used.

Memory Limit (MB)
The value of Data cache limit controls the amount of data blocks Imaris will keep in memory at any time.

File Paths:
Display of the cache file paths.

Button **Add**
You can use the buttons to add file paths in the list.

Button **Remove**
You can use the buttons to remove file paths in the list.

Output Formats

If you want to extend the list of output formats you have to check the following parameter and enter a valid license in the next step. Find your licence number in Imaris as follows: Click on the menu Help, select the menu entry Licenses and copy the license number (in the License Path at the bottom of the window, next to last enter field).

Include Image Filenames
Select this option and the output file names are assigned by using a default-naming rule.

Suffix Alphabetic
The output file inherits the input file name followed by the successive alphabetic letter.

Suffix Numeric
The output file inherits the input file name followed by the number of the output files being created (consecutive integer numbers).

All Imaris Output File Formats
Check this box to extend the output file formats.

License Location
Type in your license number or use the button **Browse**.

Button **Browse**
Click on this button and select the license path.

Button **OK**
Click on this button and all available Imaris output formats are available.

Standard formats:
Bitplane: Imaris 5.5 (*.ims)
Bitplane: Imaris 3.0 (*.ims)
Bitplane: Imaris 2.7 (Classic)(*.ims)

Additional formats:
Tiff (series)(*.tif *.tiff)
RGBA-Tiff (series)(*.tif *.tiff)
ICS file (*.ics *.ids)
Olympus: cell^R 1.1/standard (*.tif *.tiff)
Open Microscopy Environment XML (*.ome)
Open Microscopy Environment Tiff (*.tif *.tiff)
BMP (series)(*.bmp)

See also:
Menu File - Save as ...

2.8 Scene Viewer...

To start Imaris Scene Viewer from Imaris click on the **Scene Viewer** option.

2.9 Batch

To start Imaris Batch Coordinator from Imaris click on the **Batch** option.

2.10 Inport Scene ...

A Scene comprises the Surpass Tree including all existing Items. The Scene can be loaded again to the same data set or to another data set.
The Scene can be loaded as an Imaris Scene File with the extension *.**imx** or as an Imod Binary File with the extension *.**imod** and *.**mod**.
The Imod Scene, with *.**imod** and *.**mod** extensions, contains Surpass objects: **Spots**, **Surfaces** and **Contour Surfaces**.

Load Imaris Scene...

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Select the directory and requested file to be loaded, and click **OK**. The Scene File is loaded.

To adjust which objects of the Imod scene are loaded, select the **Edit** menu- **Preference**- **AdvancedParameters** – **FileLoading**.
To control which part of the imod scene is loaded, alter the setting of the parameters (true / false):
ImodSceneLoadContour
ImodSceneLoadWildContours
ImodSceneLoadSurfaces

See also:
Menu File - Export Scene...
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Scene File Concept

2.11 Export Scene

A Scene comprises the Surpass Tree including all existing Items. This Scene can be saved as an Imaris Scene File with the extension *.imx. The Scene can be loaded again to the same data set or to another data set.

Save Imaris Scene

Select the directory and enter the Scene File name and click **OK**. The Surpass Tree Items are saved as Imaris Scene File.

See also:
Menu File - Import Scene... ...
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Scene File Concept

2.12 Snapshot

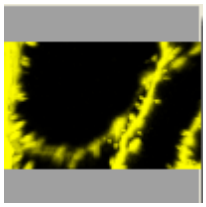
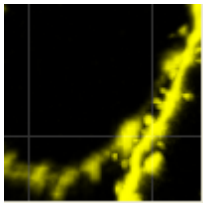
Snapshot captures a 2D image of the 2D, 3D or 4D image data as it currently appears in the viewing area. It creates a new file containing your image as well any scale information, measurements data, and ROI that are displayed. The snapshots can be (1) stored in TIFF-Format, (2) saved as database records in ImageAccess in order to manage them more efficiently or else (3) copied to the clipboard.

The Image Size settings specify the size of a Snapshot. The snapshot size (in pixels) is equal to the settings in the width/height numerical fields.
The default setting for snapshot size is the Snapshot size from window size option. To modify and customize snapshot dimensions you can either select the predefined sets from the drop-down list or manually adjust the **Width** and **Height** values.
Adjusting the width or height settings for the snapshot changes the number of pixels in the snapshot, by resampling and/or cropping/expanding the image in the viewing area to match the specified pixel dimensions. The placement of the snapshot borders (cropping or expansion) depends on the aspect ratio of the viewing area compared to the snapshot aspect ratio, and the setting called Crop to fill whole snapshot area (see below).
Changing the snapshot dimensions scales the input image, so that none of its dimensions are greater than the corresponding snapshot (output image) dimension. The scaled image is centred within the new snapshot size. In other words, the zoom of the input image is automatically scaled to better fit the available pixels in the snapshot. Depending on the settings used, some of the background color might also be considered to be part of the input image.
When the aspect ratio of the viewing area does not match the aspect ratio of the snapshot, the snapshot is surrounded with bars in the color of the Imaris screen background. There are two options allowing the user to adjust the content of the snapshot and to eliminate the Imaris screen background. The image can be either scaled to fit the selected snapshot dimensions (by adjusting the zoom in the viewing area) or else the Crop to fill whole snapshot area option can be used.

Snapshot preview

Snapshot preview offers a view of the snapshot content, as it will look when finalized.

Crop to fill whole snapshot area
This option crops the image to fit the snapshot specified Width and Height values. The option sets an image so that one dimension fits the snapshots viewing area, while the overflow of the other dimension is cut off, respecting image aspect ratio.
The images below illustrate Pyramidal cell image Snapshots taken with the various settings. (1) Disparity between image size and snapshot intended dimensions, (2) Crop to fill whole snapshot area.

(1)  **(2)** 

The option is only available if the option Snapshot from window size is not selected.

Image size

Specifies the size (in pixels) of the Snapshot area.
The size of the Snapshot can be chosen from the drop-down list. The drop-down list offers a predefined set of numerical parameters defining image dimensions. The last choice in the drop-down list with the suffix dataset is a choice that represents the XY dimensions of the original dataset.

Width
Defines the Snapshot width in pixels.

Height
Defines the Snapshot height in pixels.

Lock Aspect Ratio Button
This setting locks the aspect ratio when the image width/height is manually changed. It changes the height and width settings in relation to one another so that the ratio of the image's width and height remains constant.

Resolution
Resolution (dpi) controls the default size of the Snapshot when it is prepared for printing in an external program. For example, if the snapshot width and height are 512 x 512 pixels, and the resolution value is set to 100 dpi, then the default print size will be 5.12 inches x 5.12 inches when the snapshot is loaded into an external application to prepare the image for printing.

Zoom in Snapshot
The Zoom in Snapshot value displays the zoom value in the finished snapshot.

Note: The Zoom in Snapshot value is set to 1 if the option 100% Snapshot button is activated.

Snapshot size from window size
When this option is selected, the dimensions of snapshot equal the dimensions of the viewing area. (The snapshot width/height in pixels corresponds exactly to the viewing area width/height). Resizing the view area alters the snapshot dimensions.
Manually changing the Image dimensions will disable the Snapshot size from window size option.
Re-Selecting this option changes the size of the Snapshots accordingly.

100% Snapshot button

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Use of the 100% Snapshot button is an easy way to ensure that multiple snapshots have the same scale factor (pixel/voxel ratio), as it always results in snapshots with the zoom factor set to 1 pixel per voxel. By selecting the 100% Snapshot button, the width and height of the snapshot are adjusted (while maintaining the aspect ratio) to set the Zoom in the snapshot equal to 1.
This standardized snapshot scaling provides the opportunity to handle, compare and superimpose different snapshots. It can also be a useful guideline for creating snapshots where the number of pixels is neither too many nor too few relative to the detail contained in the original dataset.

Image Output

To create the snapshot, first the Image Output format must be selected. Select the Snapshot Format from Image Output type boxes. Multiple selections are possible.

Save to File
When this option is selected, clicking on the Do Snapshot! button saves the snapshot to the file and folder specified in the snapshot name field. You can either accept the automatic naming suggestion or enter a new name for the image the name field.

Copy to Clipboard
When this option is selected, clicking on the **Do Snapshot!** button copies the snapshot to the clipboard. Open another application and use the paste function.

Add to ImageAccess Database
Make sure that the database ImageAccess is started. In ImageAccess, select the requested directory and enter a name for the image. Then in Imaris click on the **Do Snapshot!** button and the image is saved on the disk and an entry is added to the database.

Snapshot name
This field specifies under which folder and filename the snapshot will be saved. Initially, the automatic naming suggestion is displayed. Snapshots are automatically placed in the same directory with the same name as the original dataset and a sequential number is inserted at the end of the name. This 3-digit trailing number will automatically increment by one each time you click Do Snapshot!, to save the user the trouble of manually typing a new filename each time.

Do Snapshot! button
Click on the Do Snapshot! button to create the image snapshot.

See also:
Menu Edit - Copy Snapshot Image
Menu Edit - Preferences - Display - Off Screen Rendering
Toolbars - Main Toolbar - Snapshot

2.13 Exit

Terminates Imaris and returns to the desktop.

3 Menu Edit

Undo	Ctrl + Z
Copy Snapshot Image	Ctrl + C
Image Properties...	Ctrl + I
Show Display Adjustment	Ctrl + D

InPress	Ctrl + Shift + P
Add Time Points...	
Delete Time Points...	
Add Channels...	Ctrl + Shift + A
Delete Channels...	Ctrl + Shift + B
Add Slices...	
Delete Slices...	
Crop Time...	
Resample Time...	
Crop 3D...	Ctrl + W
Resample 3D...	
Change Data Type...	
Preferences...	Ctrl + P
File Type Associations...	

See also:
Addendum - Mouse & Keyboard Mac

3.1 Undo

The Undo command reverses the last action, when necessary. It supports multiple undo actions, starting with the most recent one and working backwards in sequence.
All actions in the Draw , Edit and Edit Tracks tabs as well as the image processing functions can be undone by selecting the Undo command or by pressing **Ctrl+Z**.
The number of any previously applied command that can be Undone/Redone is defined in the Edit menu-Preferences-Calculations-**Maximum Number of Commands in History** filed. By default, the number of steps is 4.

3.2 Redo

The Redo command repeats the last action, if appropriate. The command supports multiple redo actions, starting with the most recent one and working backwards in sequence.

All action in the Edit, Edit tracks, and Draw tabs as well as image processing functions can be redone by selecting the Redo command or by pressing **Ctrl+Y**.

The number of any previously applied command that can be Undo/Redo is defined in the Edit menu-Preferences-Calculations-**Maximum Number of Commands in History** filed.

3.3 Copy Snapshot Image

When an image is ready to be stored, make sure that it is fully visible on the screen and not obstructed by any other windows or displays. To save the image, Imaris reads from the internal buffer, so other objects on the screen would appear superimposed on the image.

- Select Edit - Copy Snapshot Image and the image is copied to the clipboard.

Open another application and select the paste function. The image is pasted into the new application.

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See also:
Menu File - Snapshot

3.4 Image Properties...

Geometry
Data Set
Channels
(Channel 1...n)
Thumbnail
Parameters

When opening a data set, the following parameters should be checked or modified:

- Name and Description (in Data Set).
- Voxel Sizes (in Geometry).
- Channel Colors (in Channel 1...n).

3.4.1 Geometry

The geometrical settings of the actual data set are displayed.

Type

Data Type
Display of the image type.

Size

Size X, Y, Z, T
Display of the image size.

Coordinates

Voxel Size, X, Y, Z
The voxel sizes directly influence the views because they control the height of the image relative to its width. Check the parameters and adjust the **Voxel Size** and/or other settings if necessary.

Min, X, Y, Z
The minimum value of the coordinate axes.

Max, X, Y, Z
The maximum value of the coordinate axes.

Unit, nm, um, mm, m, unknown
Here you can select the unit.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

Time Point

First Box
Select the time point.

Date
Enter the collection data.

Time
Enter the collection time.

Button **All Equidistant...**
If the data set is a time series, enter the date/time for each time point or click **All Equidistant** to open the Set Equidistant Time Points dialog box.

- Enter the **Start Date** and **Start Time** and the **Time Interval**.
- Click **OK** when finished. Imaris will calculate the time for each time point in the series.

The data set must be saved to retain the changes. Click **OK** when finished or select another heading for further adjustments.

Button **OK**
To apply the changes click on **OK**.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.4.2 Data Set

Name

Data field to type in a data set name.

Description

Data field to type in a data set description.

Numerical Aperture (N.A.)

Reads out the numerical aperture (as defined in the menu Edit - Image Properties... - Parameters).

Log

Display of processing steps.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.4.3 Channels

There is no parameter on this card.

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3.4.4 Channel 1...n

In the Index of the Image Properties box click the **Channel** entry (Channel 1, Channel 2 etc.) to select the required channel.

Name

Data field to type in the channel name.

Description

Data field to type in a channel description.

Emission Wavelength

Reads out the emission wavelength.

Excitation Wavelength

Reads out the excitation wavelength.

Pinhole Radius

Reads out the pinhole radius.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.4.4.1 Tab Base Color

Red, Green, Blue
Reads out the assigned color. To change the color either adjust the values or move the square in the color circle.

To apply the changes click on **OK**.

3.4.4.2 Tab Mapped Color

Selected Color

Button **Edit...**
Click on a square in the grid and click on the button **Edit...** . Select a color and click on **OK**. The selected color is displayed in the square.

Button **Copy**
Click on a square in the grid and then on the button **Copy** to copy the color.

Button **Paste**
Click on a square in the grid and then on the button **Paste** to paste the color.

Interpolation

Colorspace
RGB
The RGB color model is an additive model in which red, green and blue are combined in various ways to reproduce other colors.

HSV
The HSV (Hue, Saturation, Value) model, defines a color space in terms of three constituent components.

- Hue, the color type.
- Saturation, the vibrancy of the color.
- Value, the brightness of the color.

Button **Interpolate**
Select two squares in the grid and click on the button **Interpolate**. The colors between the two selected squares are interpolated.

Color Table File

Button **Import ...**
Imaris comes with a set of pre-defined color tables. Click on **Import...** to open the Import Color Table File window. Select a color table file and click on **OK**.

Button **Export...**
Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

To apply the changes click on **OK**.

3.4.4.3 Tab Coloc Statistics

The tab Coloc Statistics is available when a data set contains a Coloc channel and the Coloc channel is selected in the channel selection of the Image Properties.

All statistics about the resulting colocalized volume are displayed. Definitions of the displayed values and further information are given in chapter Coloc - Volume Statistics.

Button **Export...**
Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

See also:
Coloc - Volume Statistics

3.4.5 Thumbnail

Type

None
Select None if no thumbnail should be displayed.

Middle Slice
Thumbnail is the middle slice.

MIP
Thumbnail in the display mode Maximum Intensity Projection. A Maximum Intensity Projection is a computer

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visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

Blend
Thumbnail in blend projection. Blends all values along the viewing direction and includes their transparency.

Preview

Displays a preview of the thumbnail image.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.4.6 Parameters

A set of informational parameters is appended to the image file.

Button **Add Group...**
Opens a dialog to add a new group.

Button **Delete Group...**
Deletes a group.

Button **Add Parameter...**
Opens a dialog to add a new parameter to the group.

Button **Delete Parameter...**
Deletes a parameter.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.5 Show Display Adjustments

The Display Adjustment function lets you choose the channel visibility as well as improve the image display by concentrating on a limited color contrast range of voxels. Usually the color contrast values of the voxels stretch over a wide range (e.g. 0 - 255).

The Display Adjustment function lets you set an upper limit for maximum color and a lower limit for minimum color (i.e., black). The range between these two limits is then extrapolated in a linear mode to the full data set range and the new voxel values are calculated.

Display Adjustment Dialog (one for each channel)

Switch the individual channels on or off.
- Check or uncheck the required channel check-box to switch the channel visibility.

Change the channel parameters such as name, color and description.

- Click on the channel name to open the Image Properties. For a detailed description please refer to chapter Menu Edit - Image Properties - Channels 1...n.

Button **Advanced**
Click on the button to open the Advanced settings (see below).

Select all Channels
Check Select all Channels to apply the settings to all channels.

Advanced Settings

Min
Max

- Enter direct values in the Min (lower limit for minimum color) and Max (upper limit for maximum color) fields.
- Alternatively drag in the display adjustment dialog the upper or lower handle of the adjustment line to adjust the Min and Max limits.

The effect of the change can be seen on the channels (channels appear brighter or darker).

Gamma
The default value of the gamma correction is 1 (the range between lower and upper limit is extrapolated in a linear mode to the full data set range). Enter a value below 1 and the linear mode is transferred to a nonlinear mode, the lower intensities appear brighter. The effect of the change is directly visible in the viewing area.

- Enter the value in the respective field.
- Alternatively click onto the middle triangle in the display adjustment dialog and drag it to the left to increase brightness/to the right to decrease brightness.

Opacity
The blend opacity adjustment allows you to change the opacity in real-time in blend projections in Section, Full 3D, and Surpass Volume views.

- Drag the blend opacity slider bar to adjust the blend opacity.
  Moving the slider to the left reduces the blend opacity, moving it to the right increase blend opacity.
- Alternatively right-click in the display adjustment dialog and drag the mouse to adjust the values.

The effect of the change can be seen on the channels (channels appear more or less transparent).

List of Shortcuts in the Display Adjustment Dialog

Please refer to chapter Menu Edit - Show Display Adjustments - Mouse & Keyboard PC.
Please refer to chapter Menu Edit - Show Display Adjustments - Mouse & Keyboard Mac.

Button **Reset**
Click the Reset button to set the image back to the original values.

Button **Auto**
When clicking the button **Auto** the system detects the real high and low values (e.g. 10 - 150) and sets the Max. and Min. limits automatically to these values.
If you check the parameter Select all Channels (see above) all channels are calculated consecutively.

Button **Auto Blend**
This button is useful if you display your data in the Blend mode (item Volume - tab Settings - Mode Blend).
Click on this button and Imaris automatically calculates the optimized Min. and Max. limits. A good portion of the selected image channel becomes transparent.
If you check the parameter Select all Channels (see above), all channels are calculated consecutively.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

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Histogram

A histogram is a statistical data analysis, representing linear voxel within an image of the selected channel.

Change Channel Color

Click on the channel name to switch directly to the channel properties (Menu Edit - Image Properties - Channels 1...n).

See also:
Menu Edit - Image Properties... - Channel 1...n
Menu Edit - Show Display Adjustments - Mouse & Keyboard PC
Menu Edit - Show Display Adjustments - Mouse & Keyboard Mac

3.5.1 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Display Adjustments Window

Ctrl + D	Shows the Display Adjustment window
Left-click	Select channel
Left-click on middle triangle and drag	Adjust Gamma Correction
Ctrl + left-click	Add channel to selection, or remove channel from selection
Left-click & drag	Move left: make image channel brighter Move right: make image channel darker Move up: increase image channel contrast Move down: decrease image channel contrast
Right-click & drag	Move left: make image channel transparent Move right: make image channel opaque
Ctrl + right-click	Automatic range for Min and Max

See also:
Addendum - Mouse & Keyboard PC

3.5.2 Mouse & Keyboard Mac

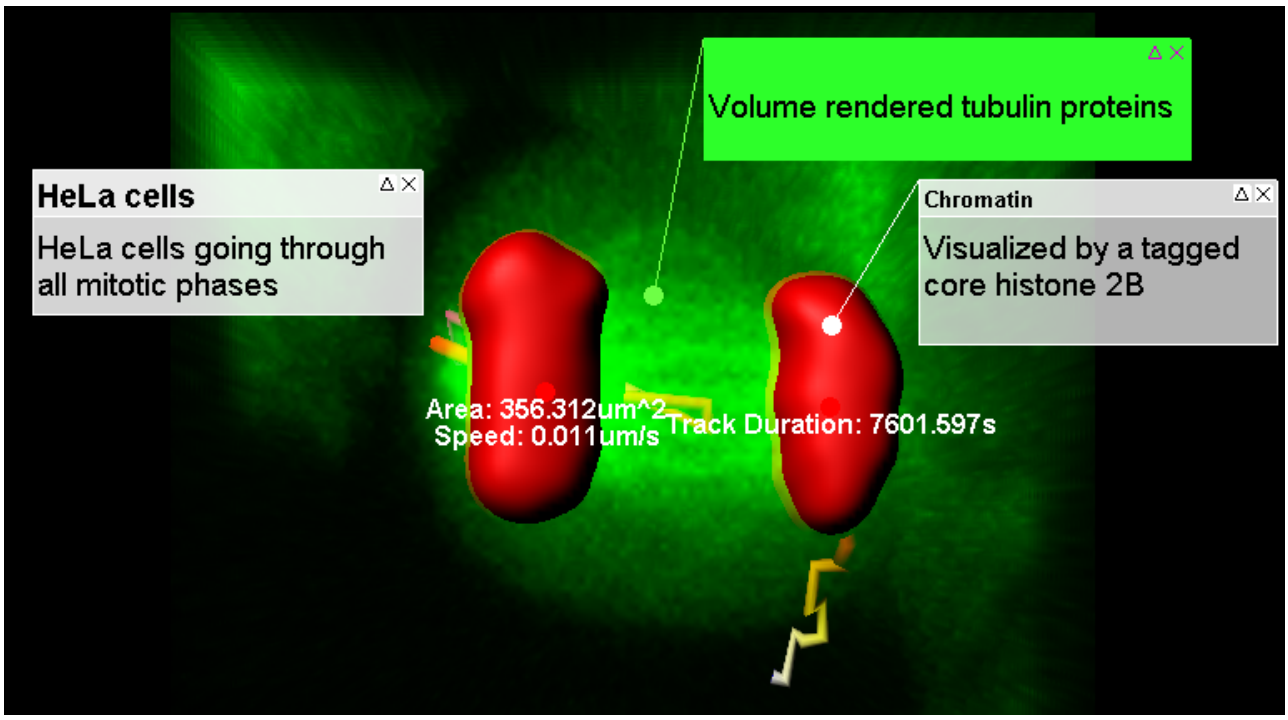
Mouse & Keyboard Functions in the Display Adjustments Window

Command + D	Shows the Display Adjustment window
Click	Select channel
Left-click on middle triangle and drag	Adjust Gamma Correction
Command + click	Add channel to selection, or remove channel from selection
Click & drag	Move left: make image channel brighter Move right: make image channel darker Move up: increase image channel contrast Move down: decrease image channel contrast
Ctrl + click & drag	Move left: make image channel transparent Move right: make image channel opaque
Command + Ctrl + click	Automatic range for Min and Max

See also:
Addendum - Mouse & Keyboard Mac

3.6 InPress

InPress allows you to create figures of all Imaris views ready with the selected statistical values, titles, captions, labels, and other highlights for presentations or publications.



Figures are composed of annotations or symbols, a 3D attachment site, and text.
Annotations and symbols are 2D elements which assures optimal reading.
The attachment point and the line are dynamic elements they are updated as you change the view or the camera angle.
Figures can be exported to Html.

3.6.1 Insert Figures and Annotations

To activate this module click on the Icon **InPress**, press Ctrl + Shift + P, or in the menu bar select menu File – InPress. To close Imaris InPress press the button **InPress**.
The **Figures window** appears on the right side of the screen.
The Figures window is open in all Imaris views and applies to all views.
For better handling it can also be undocked by dragging it off its integrated position or clicking on the docking icon (top right, next to x).

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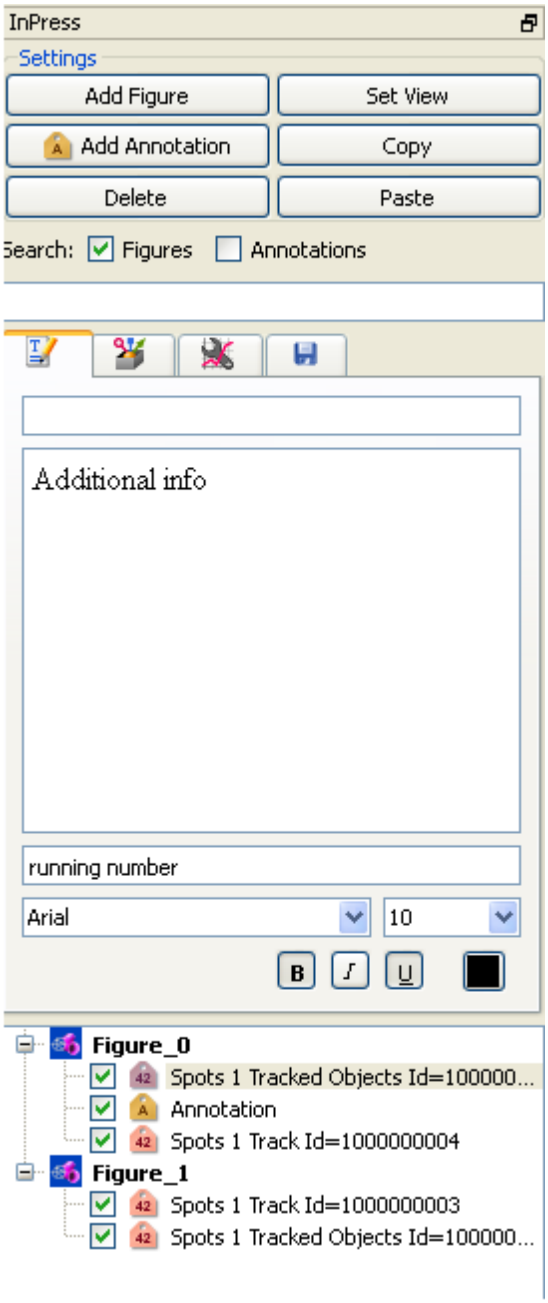
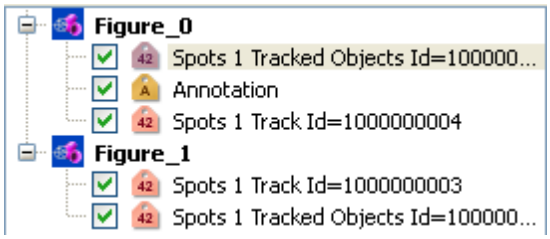


Labeling that occurs in Imaris InPress has two main parts: **Figure** and an **Annotation**.

Figure Tree

The **Figure Tree** is composed of all the existing Figures. In the Figure Tree all existing figures and annotations are displayed.



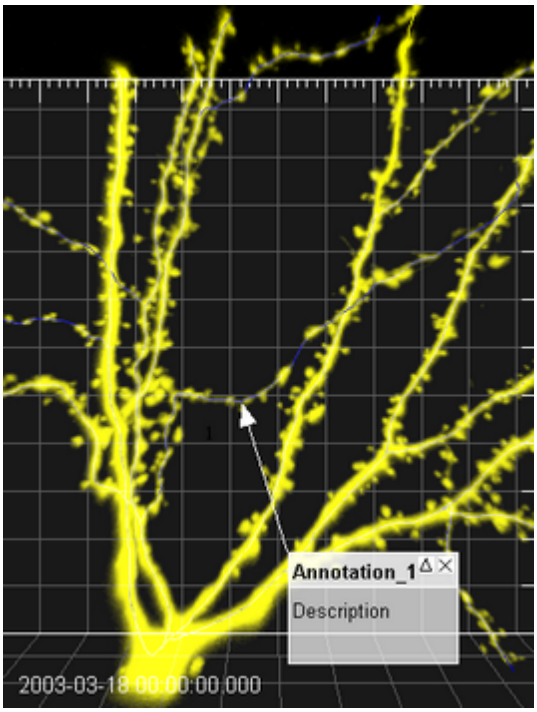
Within the selected Figure all the annotations' names are displayed.
For a Figure, Imaris remembers the view that you were in and the camera position.

Annotations

The labels that are displayed in an image are called Annotations.
In Imaris two types of the annotations exists Text annotation and Statistical annotation .
The Statistical annotation icon indicates the type of the selected statistical value and for that reason your chose of the selected statistical value type will affect the appearance of the statistical annotation icon.
The following options are available:
Selecting a **statistical variable** that **changes over time** inserts the Statistical Annotation with the **Time icon** in the selected Figure.
Selecting a **statistical variable** that is **not time dependent** inserts the Statistical Annotation icon in the selected Figure.
Selecting or de-selecting the box next to the annotation name in the Figure Tree controls the visibility of the annotation in the viewing area.

See also:
Menu File - Export Scene...

3.6.2 Annotation Box



Text Annotation

To create Text annotation in the **Setting** options select the **Add Annotation** button Add Annotation.
To create Text annotation box click in the image to set the 3D anchor point.
Move the mouse to elongate the line. With the second click you fix the top left corner of the text box.
Move the mouse to adjust the text box size. With the third click you fix the text box size.

Statistical Annotations

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By default, **statistical annotations** are presented in the viewing area without an annotation box. To create the annotation box for the **statistical annotations** select the annotation in the Figure Tree, and then activate the **Text box** option under the Style tab .

The position of the statistical annotations can be adjusted by moving the annotation box and the anchor area.

The statistical annotation is assigned to the selected objects in the image and the anchor point cannot be placed on another object.

Once created the annotation box and anchor point can be moved to the preferred location.

Move Annotation Point, Box, Number

To move the **3D anchor point** set the mouse over the anchor point (until it changes to a cross) then click & drag to a new location.
To move the **annotation box** set the mouse on the upper region of the box (until it changes to a cross) then click & drag to a new location.
To move the **annotation number** set the mouse over it (until it changes to a cross) then click & drag to a new location.

Resize the Annotation Box

To resize the annotation box set the mouse to an edge of the box (until the mouse changes to a resize icon) and then click & drag the edge.

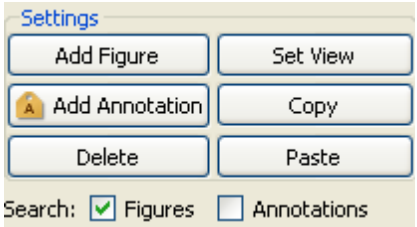
Annotation Visibility

The description area of the annotation can be closed (hidden) or expanded (shown) by clicking the triangle on the top right of any annotation box.
The annotation can be hidden by clicking the "X" on the top right corner of the box.
Selecting or de-selecting the box next to the annotation name in the Figure tree controls the visibility of the annotation in the viewing area.

Save Figures and Annotations in Scene File

The figure legends are saved in the scene file of a data set.

3.6.3 Settings



The first buttons in the left row are to **Add Figure**, **Add Annotation** and **Delete** selected figures/annotations.
Click on the button **Set View** you can save the actual image view for the selected figure.
Click on the button **Copy** to copy the selected figure/annotation.
Click on **Paste** to paste the copied figure/annotation.

Create a Figure

Click on Add Figure. In the first row of the tab Text the standard text header Figure_0 is displayed. To name a figure type the name in the text box.
In the second row you can add a figure description. A figure label appears in the Imaris InPress Tree.

To jump to the view and camera position associated with a figure double-click the figure name in the Figure Tree. To change the view associated with a particular figure, highlight the name of the figure in the list, move to the Imaris view of interest, and press the button **Set View** from the Settings of Imaris InPress

How to Create Text Annotations

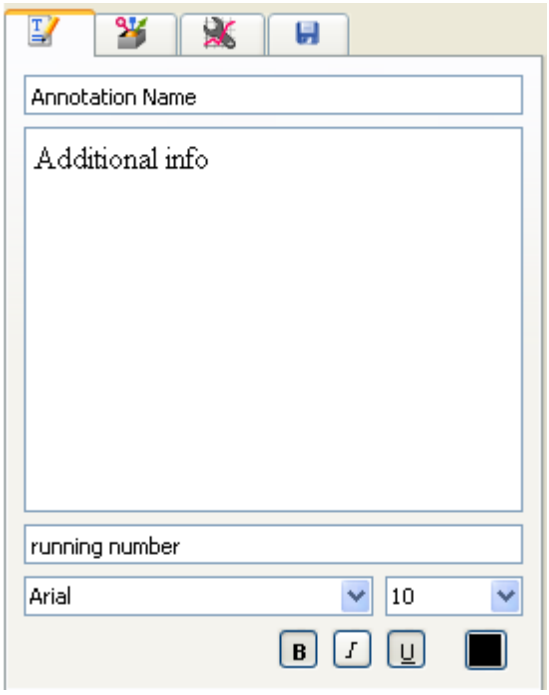
Annotations can be added to any figure you want to create.
First you have to select the Figure name in the Figure Tree.
The Figure name is highlighted.
Click on Add Annotation. In the first row of the tab Text the standard text header Annotation_0 is displayed.
To edit the annotation title select the Text tab . In the Annotation title description window write new description.
In the second row you can add an annotation text.

Search for Figures and Annotations

You can search for the initials in the title field (first row on the tab Text) of a Figure or Annotation.

3.6.4 TabText

The Text tab allows you to modify the object label, insert an additional description, and change the font type, size and color.



Title

The top line controls the annotation title field.
The title for statistical annotations is by default the object name, category and ID number.

Additional description

Displays the current text of the annotation controls the description field (text). Edit this field to change the annotation text.

Description running number

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The third box by default contains the annotation running number (legend). To remove the number from the image, delete it from the text field

Font

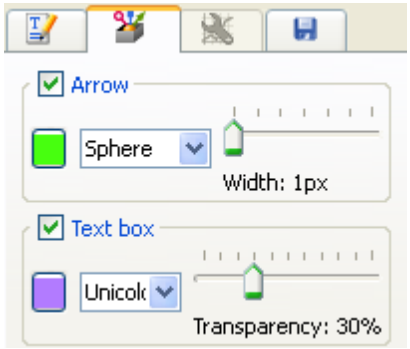
Select this option to adjust the appearance of the text.
The parameter specifies the name of the font to use for the text. This font must be supported on your system, in order to be displayed and printed properly. The default font is Arial. This value specifies the font size. The default size is 10.

All changes made in the Text Tab are applied for all time points.

To display the default settings click on the menu Edit - Preferences... and select InPress.

3.6.5 Tab Style

The **Style Tab** lists the properties you can modify on an annotation box and annotation anchor point.



Arrow

Select this option to adjust the appearance, color, style and width of the arrowhead.
In the drop-down list you select the shape of the pointer. The pointer can have three possibilities; a sphere, an arrow, or a magnifier
Width of the arrowhead can be modified by specifying this property in points.
Selecting the check-box turns on or off the Arrow.
With a click on the color selection you can change the color of the Arrow.
The slider controls the size of the line and the pointer.

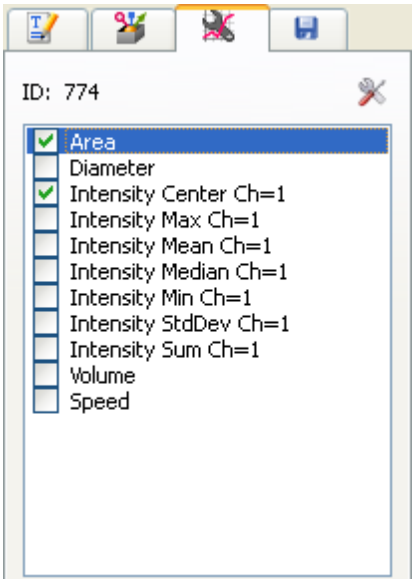
Text Box

Select this option to adjust the appearance, the background color and transparency of the annotation box.
With a click on the color selection you can change the color of the Text box.
The pointer can be colored in three ways; uni-color, black text area, or a black title area.
Transparency controls the Text box background. This property defines the degree to which the object's background color is transparent. A value of 100 makes the background completely transparent.

3.6.6 Statistical Annotations

The Statistical annotation icon indicates the type of the selected statistical value.
Selecting a **statistical variable** that **changes over time** inserts the Statistical Annotation with the **Time icon** in the selected Figure.
Selecting a **statistical variable** that is **not time dependent** inserts the Statistical Annotation icon in the selected Figure.

The statistical annotations name is automatically generated and contains as a default the object name, category and ID number.



The ID number of the selected object appears on the top of the statistical tab.

For all object types that have time dependent statistical parameters, an individual statistical annotation is created for every single time point.

Statistical annotations presents always statistical parameter value for the current time point, both in the viewing area and in the Figure Tree.
Selecting or deselecting the box next to the annotation name in the Figure tree controls the visibility of the annotation in the viewing area for all time points.

Clicking on the statistical annotation name, the annotation is highlighted and the list of statistical values appears in the Annotation window.
This list can be customized, by clicking on the **Preferences** icon.
The option allows you to choose a set of statistical values to be displayed in the viewing area, which differs from the ones selected from the Statistical table.

By default, **statistical annotations** are presented in the viewing area **without a text box**. To create the annotation box for the **statistical annotations** select the annotation in the Figure Tree, and then activate the **Text box** option under the Style tab . The text box appears in the viewing area and creates the editable annotation. Select the Text tab and in the description windows write additional details.

Preference
The list of statistical values used in the annotation table, the list can be customized.
Clicking on the icon preferences opens a new window with the list of all possible statistical values available for the selected object type.
Customization of the list of statistical values is possible by selecting the checkbox next to the statistical value name. If the box is checked then the statistical value is visible in the annotation table and if is unchecked then the value is hidden.

This section does not attempt to describe all features of the Statistical values' calculation in Imaris. For further information please read the description in the Chapter Statistics.

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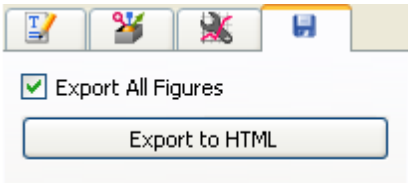
3.6.7 Tab Export



Check the check-box if you want to export all figures.
Highlight the figure in the InPress Figure Tree that you want to Export.
With a click on the button **Export to HTML** you open the Export Figure as HTML window.
Choose the desired file path, enter a file name to save the document.
A web page is created that contains the image name and location, each channel name, the number of time points, the image size, the number of slices, the voxel sizes, a snapshot of the view that is associated with the figure, and the figure name.

3.7 Add Time Points...

Additional time points can be added to the data set.

- Select the required image and click **Open**.

The time points to be added must contain the same number of channels and be of the same format as the current data set. A warning message appears if the formatting does not match.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

3.8 Delete Time Points...

Superfluous time points can be deleted.

Parameters

Delete From, To
Specify the time points to be deleted and click **OK**. The selected time points are automatically deleted from the data set.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

3.9 Add Channels...

Adding channels is used to extend or complete the data set information if a microscope can only export one channel as a file at one time. All channels of a selected data set are added. The size of the data sets must be identical.

Window: Add Channels

- Select the requested file and click **Open**.

All channels from this data set are added automatically. If the size of the data set does not match a warning message is issued.

Button Add to Batch
Add to Batch button places the command into Batch coordinator.

3.10 Delete Channels...

Deleting channels is used to erase unwanted information in a data set.

Select Channels to Delete

Channel 1...n
Select the channel to be deleted in the Delete Channels box and click **OK**. The effect is immediately visible on the image.

Button Add to Batch
Add to Batch button places the command into Batch coordinator.

See also:
Menu File - Open - Reader Configuration

3.11 Add Slices...

Adding slices may become necessary if through manipulation or during file export or formatting the data set consists of less than the originally acquired number of slices.

Window: Add Slices

- Select the requested file and click **Open**.

All slices from this data set are added automatically. The x-, and y-values of the two data sets must be identical and the same number of channels is required.

Button Add to Batch
Add to Batch button places the command into Batch coordinator.

3.12 Delete Slices...

Parameters

Slice number [1...n]
Specify the slice to be deleted and click **OK**. The slice is automatically deleted from the data set.

Button Add to Batch
Add to Batch button places the command into Batch coordinator.

3.13 Crop Time...

The total number of time points can be reduced at the beginning or end of the series of images.

Parameters

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From, To
Enter the time points to be included in the data set and click **OK**. All other time points are deleted from the data set.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

See also:
Menu File - Open - Reader Configuration

3.14 Resample Time...

The total number of time points can be reduced to display the images at a faster speed.

Parameters

New Number of Time Points
Specify the number of time points needed and click **OK**.

The process of resampling the time points takes a while. When the process is finished, the time bar displays the new number of time points.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

See also:
Menu File - Open - Reader Configuration

3.15 Crop 3D...

Cropping the data set allows you to crop the images down to the region of interest. Cropping reduces the size of the data set and makes it easier and faster to handle the viewing and storing of the images.

Preview

Display of a sectional view of the actual image (current time point). A rectangle, representing the region of interest (ROI), is overlaid on all three views.

Select Crop Dimensions

X, Y, Z, From, To, Size
Modify the size and the position of the region of interest by entering the direct values in the corresponding x-, y-, and z-fields or as follows:

- To move the ROI, click inside the rectangle, hold the mouse button down & drag the entire ROI around.
- To shape the ROI, click on a handle, hold the mouse button it down and reshape the ROI. Side handles affect one direction, corner handles two directions.

The modifications apply to all slices and all time points of the image.

Click **OK** when finished. The data set is cut down to the marked ROI. The rest is erased.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

See also:
Menu File - Open - Reader Configuration

3.16 Resample 3D...

Resampling reduces the voxel density in a data set to fasten its processing. Reducing the data size also deteriorates the resolution. Resampling reduces the number of voxels in a grid but keeps the original relationship between the voxels.

New Size

X, Y, Z
The fields display the current x-, y-, and z-values. The requested values can be directly entered in the fields.

Aspect Ratio

Fixed Ratio X/Y
Fixed Ratio X/Y/Z
The Aspect Ratio of the data set's dimensions can be kept by checking the respective Fixed Ratio options. Clicking the **OK** button resamples the data set to the entered values.

Use a Gaussian filter as low-pass before sampling down an image.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

See also:
Menu File - Open - Reader Configuration
Menu Image Processing - Smoothing - Gaussian Filter

3.17 Change Data Type...

Type

From
Displays the current data set type.

To
Drop-down list to select the requested data set type from the supported types:

- unsigned 8 bit for the range 0...255.
- unsigned 16 bit (0...65535).
- 32 bit float.

Range Adjustment

Check field to determine how the data values are translated during the change.

None
Data values are imported in the new type.

Source Range to Target Range
Maximum data values are scaled to the new range (e.g., 0...255 to 0...65535).

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Data Range to Target Range
Actual data range values are interpolated to the new range (e.g., 0...150 to 0...65535).

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

3.18 Preferences...

The adjustable parameters in the preferences are application specific and Imaris stores this preferences for an individual user.

System
Display
Loading
Calculation
Time
Surpass
Creation Parameters
Statistics
Custom Tools
Licences
Update
Usage Data
3D Cursor
InPress
Advanced

3.18.1 System

Displays the basic system parameters of your Windows computer.

Processor

Number of Processor
Processor Architecture
Processor Speed
Information about the number of Processors, Processor Type and Processor Speed.

Graphics

OpenGL Renderer
OpenGL Version
Pixel Shader
OpenGL Extensions
Information about the OpenGL Renderer, OpenGL Version, Pixel Shader, and OpenGL Extensions.

Operating System

OS
Version (Build)
Service Pack
Information about the operating system, the installed Version and the service pack.

Memory Status

Physical Memory installed
Physical Memory available
Information about the available amount of internal memory.

Network

Network Interfaces
Information about the available network interfaces

Proxy Settings
Http Proxy Server Name
Enter a proxy server's name. If this field is empty, no proxy server is used.

Http Proxy Server Port
Enter the port to the proxy server. If this field is empty, no proxy server is used.

Test Connection Button
Click on this button to test your proxy settings. If the fields are empty, the connection is tested without using a proxy server.

Button OK
To apply the changes click on **OK**.

Button Cancel
If you do not want to save the changes click on **Cancel**.

3.18.2 Display

Select the viewing properties and the basic colors for the backgrounds and selection in the gallery.

Display

Interpolate
If checked, the images are automatically interpolated for a smoother display.

Texture Cache Limit (MB)
Before displaying any image data, Imaris converts the data into a configuration (called textures) that is optimized by the graphics hardware. The value of the Texture Cache Limit determines how many textures can be stored in RAM. Set the value to the memory of your graphics card.

Colors

Background Color
The background color appears when there is no image to be displayed or when the displayed image does not fill the whole View Area.

Please note: The background color setting has not effect on the MIP mode. The default color for MIP(max) is black and MIP(min) is white.

Button **Select...**
Click on this button to open the color selection window to change the respective color.

Background Color 2
Background marking the original position if an image is moved.

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Button **Select...**
Click on this button to open the color selection window to change the respective color.

Checkered Background for Blending
When using the blending mode, a checkered background displays in Full 3D blend and in Surpass.

Tile Size
Allows definition of the tile size for a checkered background.

Linear Color Progress for Blending
The background displays a color gradient in blend progress projections and in Surpass.

Selection Color
Color of selection frame and drawing lines in contour surfaces.

Button **Select...**
Click on this button to open the color selection window to change the respective color.

Measurement Color
Color of measurements points and lines visible in the image (in the Slice view and the Surpass view).

Button **Select...**
Click on this button to open the color selection window to change the respective color.

Coordinate Axis/Scale Bar

Show Coordinates Axis
Show Date
Show Scale Bar
Show Time
Show Scale Bar Label
Show relative Time

These options change the display of the scale bar, labels and time information in the Slice, Selection, Gallery and Surpass View.
To choose the style of timestamp to be displayed select either **Show Times** or **Show relative Time.**
Show Times option display the acquisition time.
Show relative Timed
The option display timestamps for the image time series. The first time point is designated as time zero and all other times are displayed relative to that.

Select options to display coordinate axis, date, scale bar, scale bar label or time on screen in the Viewing Area.

Please note: To display the **Coordinates Axis** in the Surpass view the option Axis Labels in the Frame-Tab Setting must be also selected.

Adjusting Scale Bar
You can alter the length, width, location and font size of the interactive scale bar. All changes are instantly displayed in the viewing area.
Extend or shorten the scale bar by clicking and dragging one end of it.
Move the scale bar to any location in the viewing by dragging it
Zooming in or out of the image the scale bars are automatically updated.

Off Screen Rendering (for saving Snapshots and Movies)
Check this box to save only the viewing area. For the Snapshot and Movie Imaris hides additional control elements. If you uncheck this box the actual screen display is saved, e.g. if the window Display Adjustments is

in front of the viewing area this window is saved as well.

Show System Monitors
Check this box to display the system monitors in the Status Bar at the bottom of the screen. The first window displays the "reads per sec", the second the "writes per sec", the third the "write requests in queue" and the last the "percentage read cache hits". These are useful information especially if you work with huge data sets.

Button OK
To apply the changes click on **OK**.

Button Cancel
If you do not want to save the changes click on **Cancel**.

See also:
Menu File - Snapshot

3.18.3 Loading

Allows you to select the color assignment method used when loading data sets and to define the default colors. Images in Imaris format will display in the colors defined in the image file.

Take Colors from:

Default Colors
Use the default color selection to display the loaded data set. The parameter set Default Colors (see below) is available.

File Colors (color table or base color if available, otherwise default colors)
Use the original color definition of the loaded data set (usually stored in a lookup table). The parameter set Default Colors (see below) is not available.

Emission Wavelength (from file if available, otherwise default colors)
Use the color according to the emitted wavelength from the file (corresponds to the appearance under the microscope). The parameter set Default Colors (see below) is not available.

Please note that not all file formats support lookup tables and emission wavelength.

Default Colors

The parameter set Default Colors is available, if you select Default Colors in the parameter set Take Colors from (see above).

First Cannel
Second Channel
Third Channel
Other Channels
Display of the defined color.

iQ Disk Image File

Either type in the location of the **DisckInfo5.kinetic** file or use the browse button to find it.
Use the Browse button to find and select the DisckInfo5.kinetic file.
Open iQ files'
Clicking on the '**Open iQ files**' opens the Image Selection window with a list of images and image information.
Check the box Thumbnail Preview to activate an image preview.
Select the required image and click on the OK button.
This will instantly create a 3D volume rendered image of the selected data set.

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Browse Button
Use the Browse button to find and select the DisckInfo5.kinetic file.

Select... Button
Click on this button to open the color selection window to change the respective color.

OK Button
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.18.4 Calculation

Calculation

Number of Processors
Specify the number of processors used in calculations. See maximum number of available processors in the System box.

Image Processing History

Maximum Number of Commands in History
Defines the maximum number of operations that can be undone/redone.

Please note: Each level of image processing requires an additional copy of the full image in memory. If your machine runs out of memory, set Maximum Number of Commands in History to 1.

Data Cache

Imaris uses a data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.

Memory Limit (MB)
The value of data cache limit controls the amount of data blocks Imaris will keep in memory at any time. Enter a value based on the following table.

PC
32 bit Physical memory installed x 0,5; but not higher than 1.2 GB
64 bit Physical memory installed x 0,5

Mac
32 bit Physical memory installed x 0,5; but not higher than 2 GB

Button **Apply**
Press this button to apply the changes.

Cache File Paths:
Imaris utilize advanced cashing when working with large images. **Cache File Paths** display of the paths to the temporary files (Scratch-disks).

Please note: Setting the Cache File location to a otherwise not used disc, improves the performance when

working on larger images. This benefits a lot from a fast SSD with minimum size 10 times the biggest dataset which will be used.

Button **Add**
Button **Remove**
You can use the buttons to add or remove file paths in the list.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.18.5 Time

These are the default parameter settings for the Time Bar.

Play Back

Specify the play back mode for the Time Bar.

Play One Time
All time points of the data set are shown one time. The play back stops when the last time point is reached.

Repeat Forever
Once the play back has reached the last time point, it starts at the first time point again (never ending).

Swing Back and Forth Forever
When the last time point is reached, the time sequence is shown in reverse until the first time point is reached.

Frame Rate: ... Frames per Second
You can further specify the frame rate, i.e. the number of frames per second.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

See also:
Toolbars - Time Bar

3.18.6 Surpass

Object Creation Buttons

Check the icons to be displayed on the Objects toolbar in the Surpass View.

Button **Move Up**
Button **Move Down**
Highlight an icon and click Move Up or Move Down to define the order of the icons in the Objects toolbar.

Key Frame Interpolation

Object Rotation Center (optimizing default user interaction)

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This is the default parameter, select Object Rotation Center to create a rotated animation. The distance from the camera to the object rotation center is always the same.

Camera Rotation Center (optimizing fly through animation)
Select Camera Rotation Center to create a fly through animation. The distance from camera to object is always the same, the rotation is not around a fixed rotation center.

Key Frame Animation

Specify the play back mode for the Key Frame Animation.

Play One Time
All time points of the data set are shown one time. The play back stops when the last time point is reached.

Repeat Forever
Once the play back has reached the last time point, it starts at the first time point again (never ending).

Frame Rate ... Frames per Second
You can further specify the frame rate, i.e. the number of frames per second.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

See also:
Surpass View - Overview - Surpass Tree (Objects toolbar)

3.18.7 Creation Parameters

When you save the Creation Parameter file, it is automatically added to the list that is displayed under the Edit/Preference/Creation Parameters.

The preference window shows a list of all the creation parameters sets. To assist you in finding and organizing the creation parameters, each file is labeled with an object icon, which indicates the type of creation parameters set.

Click on a name of the creation parameters set the extended window provides an option to view the entire list of operations, processing instructions and the parameters value of the selected set.

Remove Button

This function will remove the selected set of creation parameters from the list.
The Export and Import functions provide an easy way for exchange of the creation parameters between different users or groups.

Export Button

The creation parameters files can be created and exported.
This can be achieved by selecting the creation parameters set from the list and pressing the Export button.
Specify the directory where you want to save the creation parameters file, select a name and save it.

Import Button

The creation parameters files, that can be imported, are generated by the Export command.
Specify the directory of the files you want to import and select the file from the chosen location.

3.18.8 Statistics

Show Statistic Values

The most desired set of statistics values can be specified (for display, export to MS Excel, or sorting). Check the values to be displayed when you open the tab Statistics in the Surpass View.

Show All
Check this box and all statistical values are selected. Uncheck the box and all statistical values are unselected.

For details please refer to the respective chapter:

Menu Edit - Preferences ... - Statistics - Cell
Menu Edit - Preferences ... - Statistics - Filament
Menu Edit - Preferences ... - Statistics - Measurement Points
Menu Edit - Preferences ... - Statistics - Spots
Menu Edit - Preferences ... - Statistics - Surfaces
Menu Edit - Preferences ... - Statistics - Volume

Button OK
To apply the changes click on **OK**.

Button Cancel
If you do not want to save the changes click on **Cancel**.

See also:
Surpass View - Overview - Properties Area (Tab Statistics)
Coloc - Volume Statistics

3.18.8.1 Cells

ImarisCell is an Imaris module that performs automated 2D and 3D time-resolved cell image analysis. It extracts a full spectrum of relevant information, present in the dataset, which provides a wide range of options to analyze 3D and 4D image data.
ImarisCell detects some features that are not easily detected by a human observer, which can lead to the correlation of potential important and otherwise missed variations in the cell structure and function.
Cells statistics are automatically computed for each Cell object.

Show Statistic Values - Cell

Cells - Cell Acceleration
Acceleration is the change in the Cell velocity over time.

Cells - Cell Acceleration X
Cells - Cell Acceleration Y
Cells - Cell Acceleration Z
Acceleration is the change in the Cell velocity over time along the selected-axis.

Cells - Cell Area
The area is the sum of the outer voxel faces.

Cells - Cell Center of Homogeneous Mass X
Cells - Cell Center of Homogeneous Mass Y
Cells - Cell Center of Homogeneous Mass Z
The voxel intensity values are taken to be the ‘mass’ of each voxel.
Assuming equal voxel intensity, the center of homogeneous mass is the centroid of the object. Depending on

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the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in object} r_i$$

R : center of homogeneous mass (x y z)
r_i : center of a voxel (x_i y_i z_i)
n : number of i $\in$ Cell

Cells - Cell Center of Image Mass X
Cells - Cell Center of Image Mass Y
Cells - Cell Center of Image Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Image Mass is the centroid of the object calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in object} m_i * r_i$$

R : center of image mass (x y z)
m_i : voxel intensity
r_i : center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Cells- Cell Vesicle Diameter Min
The minimum diameter of all Vesicles within the Cell objects.

Cells - Cell Vesicle Diameter Mean
The mean value of diameter of all Vesicles within the Cell objects.

Cells- Cell Vesicle Diameter Max
The maximum value of diameter of all Vesicles within the Cell objects.

Cells - Cell Displacement ^2

$$\bar{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t,t-\Delta t)^2 + D_y(t,t-\Delta t)^2 + D_z(t,t-\Delta t)^2$$

t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cells - Cell Displacement ^2 X
Cells - Cell Displacement ^2 Y
Cells - Cell Displacement ^2 Z
The Cell squared displacement along the selected-axis.

Cells- Cell Distance from Vesicle to Closest Nucleus Mean

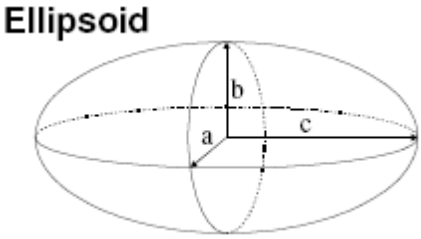
The mean distance, calculated by the distance map method, between the Vesicle's centers and the Nucleus surface border within the Cell object.

Cells- Cell Distance from Vesicle to Closest Nucleus Std.Dev.
This value describes the Standard Deviation of the distance between the Vesicle's centers and the Nucleus surface border within the Cell object.

Cells- Cell Distance from Vesicle to Membrane Mean
The mean distance, calculated by the distance map method, between the Vesicle's centers and the Cell surface border.

Cells- Cell Distance from Vesicle to Membrane Std.Dev.
This value describes the Standard Deviation of the distance between the Vesicle's centers and the Cell surface border.

Ellipsoid Axis



Cells - Cell Ellipsoid Axis A X
Cells - Cell Ellipsoid Axis A Y
Cells - Cell Ellipsoid Axis A Z
These three parameters define the Vector of the Ellipsoid Axis a.

Cells - Cell Ellipsoid Axis B X
Cells - Cell Ellipsoid Axis B Y
Cells - Cell Ellipsoid Axis B Z
These three parameters define the Vector of the Ellipsoid Axis b.

Cells - Cell Ellipsoid Axis C X
Cells - Cell Ellipsoid Axis C Y
Cells - Cell Ellipsoid Axis C Z
These three parameters define the Vector of the Ellipsoid Axis c.

Cells - Cell Ellipsoid Axis Length A
Cells - Cell Ellipsoid Axis Length B
Cells - Cell Ellipsoid Axis Length C
Cells - Cell Ellipsoid (oblate)
Cells - Cell Ellipsoid (prolate)

If we assume $a \leq b \leq c$, then when:

- a = 0 it is an **Ellipse**
- a = b = c it is a **Sphere** (three equal sides)
- $a \neq b \neq c$ it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

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- a = b < c it is a prolate Spheroid (cigar-shaped)
- a < b = c it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2+b^2} \cdot \left(1 - \frac{a^2+b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2+c^2} \cdot \left(1 - \frac{2a^2}{b^2+c^2}\right)$$

e_{oblate} = oblate Ellipsoid

Cells - Cell Intensity Center
Cells - Cell Intensity Max
Cells - Cell Intensity Mean
Cells - Cell Intensity Median
Cells - Cell Intensity Min
Cells - Cell Intensity StdDev
Cells - Cell Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Cell (Center, Max, Mean, Median, Min, StdDev and Sum).

Cells - Cell Intensity Min of Vesicles
This value describes the minimum voxel intensity of the Vesicles within the Cell object.

Cells - Cell Intensity Max of Vesicles
This value describes the maximum voxel intensity of the Vesicles within the Cell object.

Cells - Cell Intensity mean of Vesicles
This value describes the mean voxel intensity of the Vesicles within the Cell object.

Cells - Cell Nucleus to Cytoplasm Volume Ratio
The value is defined as the ratio between Nucleus volume and Cell cytoplasm volume. The Cell cytoplasm volume is calculated by subtracting the volume of the Nuclei from the whole cell object volume.

Cells- Cell Number of Cell per Time Point
This value describes the number of Cell object in the selected time point.

Cells - Cell Number of Nuclei
The number of the Nuclei objects within the selected Cell object.

Cells - Cell Number of Vesicles
The number of the Vesicle objects within the selected Cell object.

Cells- Cell Number of Tracks
This value describes the total number of the Cell's object tracks.

Cells - Cell Number of Voxels
Number of voxels within the selected Cell object.

Cells - Cell Position X

Cells - Cell Position Y
Cells - Cell Position Z
Defines the value of X, Y, Z Cell position (x y z).

Cells - Cell Relative Vesicle Position Min
Cells - Cell Relative Vesicle Position Mean
Cells - Cell Relative Vesicle Position Max
This parameter provides Min Max and Mean values of all Vesicle Relative Positions within the cell object. This value describes the Vesicle position within the cell relative to the Nucleus and Cell object's borders.
Vesicle Relative positions is the ratio between Vesicle distance from the Nucleus border and the sum of Vesicle's distances from the Nucleus and Cell border.
If the Vesicle is located within the Nucleus the value is set to 0. If the value is 1 then the Vesicle is located at the border of Cell object.

Cells - Cell Speed
The Cell speed is the instantaneous speed of the object (um/s).
The instantaneous speed in the scalar equivalent of the object velocity.

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}}{T(t) - T(t-1)}$$

S(t) = instantaneous speed

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

S(t) = instantaneous speed
PX(t) = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

$$S(0) = \sqrt{\frac{D_x(t_1 - t_0)^2 + D_y(t_1 - t_0)^2 + D_z(t_1 - t_0)^2}{T(t_1) - T(t_0)}}$$

$$D_x(t_1 t_0) = P_x(t_1) - P_x(t_0)$$

S(0) = instantaneous speed at the first time point

$$S(n) = \sqrt{\frac{D_x(t_n - t_{n-1})^2 + D_y(t_n - t_{n-1})^2 + D_z(t_n - t_{n-1})^2}{T(t_n) - T(t_{n-1})}}$$

S(n) = instantaneous speed at the last time point

Cells - Cell Sphericity
Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, Ψ, of a particle is the ratio of the Cell surface area of a sphere (with the same volume as the given particle) to the Cell surface area of the particle:

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$$\Psi = \frac{\pi^{1/3}(6V_p)^{2/3}}{A_P}$$

Vp = volume of the particle
Ap = surface area of the particle

Cells- Cell Time Index
The value is only available for the time series data sets. The number indicates the current time point in the series for the selected object.

Cells - Cell Total Number of Voxels
Total number of voxels within the data set.

Cells- Cell Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Cells- Cell Track AR1 X
TrackAR1X is the AR1 coefficient of the model for the x-positions.
For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t,t-1) - \bar{D}_x) \cdot (D_x(t+n,t+(n-1) - \bar{D}_X)$$

t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t,t-1)$$

t_L = last time index of track
t_F = first time index of track

Cells- Cell Track AR1 Y
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

Cells- Cell Track AR1 Z
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

Cells-Cell Track Area Mean
The mean of the triangle Cell object surfaces along the track.

Cells - Cell Track Center of Homogeneous Mass X
Cells - Cell Track Center of Homogeneous Mass Y
Cells - Cell Track Center of Homogeneous Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The voxel intensity values are taken to be the 'mass' of each voxel.
Assuming equal voxel intensity, the center of homogeneous mass is the centroid of the object. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in object} r_i$$

R : center of homogeneous mass (x y z)
r_i : center of a voxel (x_i y_i z_i)
n : number of i $\in$Surfaces

Cells - Cell Track Center of Image Mass X
Cells - Cell Track Center of Image Mass Y
Cells - Cell Track Center of Image Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Image Mass is is the centroid of the object calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in object} m_i * r_i$$

R : center of image mass (x y z)
m_i : voxel intensity
r_i : center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Cells - Cell Track Displacement Length
The Track Displacement Length is the distance between first and last Cell's position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cells - Cell Track Displacement X
Cells - Cell Track Displacement Y
Cells - Cell Track Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Cells - Cell Track Duration
The Track Duration is the duration between the first and last time point within the track.

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$$\text{Duration} = \mathrm{T}(\mathrm{t}_L) - T(t_F)$$

Duration = TrackDuration
T(t) = time in seconds at time point t
t_L = last time index of track
t_F = first time index of track

Cells - Cell Track Ellipsoid Axis AX Mean
Cells - Cell Track Ellipsoid Axis AY Mean
Cells - Cell Track Ellipsoid Axis AZ Mean
These three parameters define the Vector of the track Ellipsoid Axis a.

Cells - Cell Track Ellipsoid Axis BX Mean
Cells - Cell Track Ellipsoid Axis BY Mean
Cells - Cell Track Ellipsoid Axis BZ Mean
These three parameters define the Vector of the track Ellipsoid Axis b.

Cells - Cell Track Ellipsoid Axis CX Mean
Cells - Cell Track Ellipsoid Axis CY Mean
Cells - Cell Track Ellipsoid Axis CZ Mean
These three parameters define the Vector of the track Ellipsoid Axis c.

Cells - Cell Track Ellipsoid Axis Length A Mean
Cells - Cell Track Ellipsoid Axis Length B Mean
Cells - Cell Track Ellipsoid Axis Length C Mean
These three parameters define the length of the Ellipsoid Axis a, b, and c.

Cells - Cell Track Ellipsoid Oblate A Mean
This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

Cells - Cell Track Ellipsoid Prolate A Mean
This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

Cells - Cell Track Intensity Center Mean
This value describes the mean voxel intensity in the center of the Cell along the tracks.

Cells - Cell Track Intensity Max
This value describes the maximum voxel intensity of the Cell along the tracks.

Cells - Cell Track Intensity Mean
This value describes the mean voxel intensity of the Cell along the tracks.

Cells - Cell Track Intensity Median
This value describes the median voxel intensity of the Cell along the tracks.

Cells - Cell Track Intensity Min
This value describes the minimum voxel intensity of the Cell along the tracks.

Cells - Cell Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Cell along the tracks.

Cells - Cell Track Intensity Sum
This value describes the sum of voxel intensity of the Cell along the tracks.

Cells - Cell Track Length

The Track Length is the total length of displacements within the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}$$

L = TrackLength
t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

Cells- Cell Track Number of Cells
The number of the all Cell objects along the tracks.

Cells- Cell Track Number of Nuclei Mean
The mean number of Nuclei objects along the track.

Cells-Cell Track Number of Vesicle Mean
The mean number of Vesicle objects along the track.

Cells - VesicleTrack Number of Branches
The number of track branches.

Cells - Vesicle Track Number of Fusions
The number of track fusions.

Cells - Cell Tracks Position Mean X
Cells - Cell Tracks Position Mean Y
Cells - Cell Tracks Position Mean Z
Defines the mean value of X, Y, Z track positions.

Cells - Cell Track Speed Max
The maximum value of the object's speed on the track.

Cells - Cell Track Speed Mean
The Average value of the object's speed on the track. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

Cells - Cell Track Speed Min
The minimum value of the object's speed on the track.

Cells - Cell Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Cells - Cell Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

σ- Standard Deviation
μ-Mean value

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Cells-Cell Track Sphericity Mean
Describes the mean Cell object Sphericity along the tracks.

Cells - Cell Track Straightness

$$S = \frac{D}{L}$$

S = TrackStraightness
D = TrackDisplacement
L = TrackLength

Cells- Cell Total Number of Cell
This value describes the total number of Cell objects.

Cells - Cell Track Volume Mean
The mean volume of all Cell objects along the tracks.

Cells - Cell Velocity X
Cells - Cell Velocity Y
Cells - Cell Velocity Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Δt) at time t + Δt, can be computed as the derivative of the position:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Cells - Cell Volume
Volume is quantification of how much space a Cell object occupies.

Cells- Nucleus Acceleration
Acceleration is the change in the Nucleus velocity over time.

Cells - Nucleus Acceleration X
Cells - Nucleus Acceleration Y
Cells - Nucleus Acceleration Z
Acceleration is the change in the Nucleus velocity over time along the selected-axis.

Cells - Nucleus Area
The area is the sum of the outer Nucleus voxel faces.

Cells - Nucleus Center of Homogeneous Mass X
Cells - Nucleus Center of Homogeneous Mass Y
Cells - Nucleus Center of Homogeneous Mass Z
The voxel intensity values are taken to be the ‘mass’ of each voxel.
The voxel intensity values are taken to be the ‘mass’ of each voxel.
Assuming equal voxel intensity, the center of homogeneous mass is the centroid of the object. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

R : center of homogeneous mass (x y z)

r_i : center of a voxel (x_i y_i z_i)
n : number of i $\in$ Nucleus

Cells - Nucleus Center of Image Mass X
Cells - Nucleus Center of Image Mass Y
Cells - Nucleus Center of Image Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Image Mass is is the centroid of the object calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in object} m_i * r_i$$

R : center of image mass (x y z)
m_i : voxel intensity
r_i : center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Cells - Nucleus Displacement^2

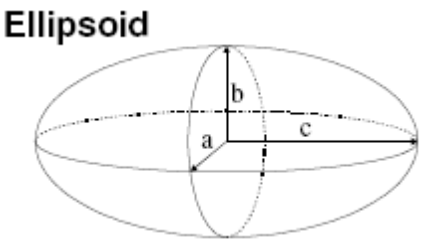
$$\bar{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t,t-\Delta t)^2 + D_y(t,t-\Delta t)^2 + D_z(t,t-\Delta t)^2$$

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

t_L = last time index of track
t_F = first time index of track
$P_x(t)$ = x-position of object at time index t

Cells - Nucleus Displacement ^2 X
Cells - Nucleus Displacement ^2 Y
Cells - Nucleus Displacement ^2 Z
The Nucleus squared displacement along the selected-axis.

Ellipsoid Axis



Cells - Nucleus Ellipsoid Axis A X
Cells - Nucleus Ellipsoid Axis A Y
Cells - Nucleus Ellipsoid Axis A Z
These three parameters define the Vector of the Ellipsoid Axis a.

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Cells - Nucleus Ellipsoid Axis B X
Cells - Nucleus Ellipsoid Axis B Y
Cells - Nucleus Ellipsoid Axis B Z
These three parameters define the Vector of the Ellipsoid Axis b.

Cells - Nucleus Ellipsoid Axis C X
Cells - Nucleus Ellipsoid Axis C Y
Cells - Nucleus Ellipsoid Axis C Z
These three parameters define the Vector of the Ellipsoid Axis c.

Cells - Nucleus Ellipsoid Axis Length A
Cells - Nucleus Ellipsoid Axis Length B
Cells - Nucleus Ellipsoid Axis Length C
Cells - Nucleus Ellipsoid (oblate)
Cells - Nucleus Ellipsoid (prolate)

If we assume $a \leq b \leq c$, then when:

- a = 0 it is an **Ellipse**
- a = b = c it is a **Sphere** (three equal sides)
- $a \neq b \neq c$ it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

- a = b < c it is a prolate Spheroid (cigar-shaped)
- a < b = c it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2+b^2} \cdot \left(1 - \frac{a^2+b^2}{2c^2}\right)$$

$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2+c^2} \cdot \left(1 - \frac{2a^2}{b^2+c^2}\right)$$

e_{oblate} = oblate Ellipsoid

Cells - Nucleus Intensity Center
Cells - Nucleus Intensity Max
Cells - Nucleus Intensity Mean
Cells - Nucleus Intensity Median
Cells - Nucleus Intensity Min
Cells - Nucleus Intensity StdDev
Cells - Nucleus Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Nucleus (Center, Max, Mean, Median, Min, StdDev and Sum).

Cells- Nucleus Number of Tracks
This value describes the total number of the Nucleus object tracks.

Cells- Number of Nucleus per Time Point
This value describes the number of Nucleus object in the selected time point.

Cells- Nucleus Number of Vesicles
The number of the Vesicle objects within the selected Nucleus object.

Cells-Nucleus Number of Voxels
Number of voxels within the selected Nucleus object.

Cells- Nucleus Position X
Cells- Nucleus Position Y
Cells- Nucleus Position Z
Defines the value of X, Y, Z Nucleus position (x y z).

Cells - Nucleus Relative To Cell Acceleration
Acceleration is the change in the Nucleus velocity over time taking into account the relative Nucleus position.

Cells- Nucleus Relative To Cell Acceleration X
Cells - Nucleus Relative To Cell Acceleration Y
Cells - Nucleus Relative To Cell Acceleration Z
Acceleration is the change in the Nucleus velocity over time along the selected-axis, taking into account the relative Nucleus position.

Cells - Nucleus Relative To Cell Displacement^2
The Nucleus squared displacement is calculated taking into account the relative Nucleus position.

$$\bar{D}^{2}(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t,t-\Delta t)^2 + D_y(t,t-\Delta t)^2 + D_z(t,t-\Delta t)^2$$

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cells - Nucleus Relative To Cell Displacement^2 X
Cells - Nucleus Relative To Cell Displacement^2 Y
Cells - Nucleus Relative To Cell Displacement^2 Z
The Nucleus squared displacement along the selected-axis, taking into account the relative Nucleus position.

Cells - Nucleus Relative To Cell Position X
Cells - Nucleus Relative To Cell Position Y
Cells - Nucleus Relative To Cell Position Z
The relative Nucleus position is calculated by subtracting the absolute position of the selected Nucleus from the absolute center position of the parent cell object.

Cells - Nucleus Relative To Cell Speed
The relative speed of the Nucleus is calculated taking into account the Nucleus relative position.

S(t) = instantaneous speed

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$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

PX(t) = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

$$S(0) = \sqrt{\frac{D_x(t_1 - t_0)^2 + D_y(t_1 - t_0)^2 + D_z(t_1 - t_0)^2}{T(t_1) - T(t_0)}}$$

$$D_x(t_1 t_0) = P_x(t_1) - P_x(t_0)$$

S(0) = instantaneous speed at the first time point

$$S(n) = \sqrt{\frac{D_x(t_n - t_{n-1})^2 + D_y(t_n - t_{n-1})^2 + D_z(t_n - t_{n-1})^2}{T(t_n) - T(t_{n-1})}}$$

$$D_x(t_n t_{n-1}) = P_x(t_n) - P_x(t_{n-1})$$

Cells -Nucleus Relative To Cell Track Displacement Length
The Nucleus Relative To Cell Track Displacement Length is the distance between first and last Nucleus's relative position.

$$D_x(t_n,t_{n-1}) = \sqrt{D_x(t_L,t_F)^2 + D_y(t_L,t_F)^2 + D_z(t_L,t_F)^2}$$

$$D_x(t_1,t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cells - Nucleus Relative To Cell Track Displacement X
Cells - Nucleus Relative To Cell Track Displacement Y
Cells - Nucleus Relative To Cell Track Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis taking into account the relative Nucleus position.

Cells - Nucleus Relative To Cell Velocity X
Cells - Nucleus Relative To Cell Velocity Y
Cells - Nucleus Relative To Cell Velocity Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Δt, can be computed as the derivative of the relative Nucleus position along the selected-axis:

Cells - Nucleus Speed
The Nucleus speed is the instantaneous speed of the object (um/s).

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}}{T(t) - T(t-1)}$$

S(t) = TrackSpeed

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

PX(t) = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

$$S(0) = \sqrt{\frac{D_x(t_1 - t_0)^2 + D_y(t_1 - t_0)^2 + D_z(t_1 - t_0)^2}{T(t_1) - T(t_0)}}$$

$$D_x(t_1 t_0) = P_x(t_1) - P_x(t_0)$$

S(0) = instantaneous speed at the first time point

$$S(n) = \sqrt{\frac{D_x(t_n - t_{n-1})^2 + D_y(t_n - t_{n-1})^2 + D_z(t_n - t_{n-1})^2}{T(t_n) - T(t_{n-1})}}$$

$$D_x(t_n t_{n-1}) = P_x(t_n) - P_x(t_{n-1})$$

S(n) = instantaneous speed at the last time point

Cells - Nucleus Sphericity
Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, Ψ, of a particle is the ratio of the Nucleus surface area of a sphere (with the same volume as the given particle) to the Cell surface area of the particle:

$$\Psi = \frac{\pi^{1/3}(6V_p)^{2/3}}{A_P}$$

Vp = volume of the particle
Ap = surface area of the particle

Cells - Nucleus Time Index
The value is only available for the time series data sets. The number indicates the current time point in the series for the selected object.

Cells - Nucleus Total Number of Voxels
Total number of voxels of the Nucleus voxels within the data set.

Cells - Total Number of Nucleus
This value describes the total number of Nucleus objects.

Cells - Nucleus Total Number of Tracks
This value describes the total number of the Nucleus object tracks.

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Cells - Nucleus Total Number of Voxels
Total number of Nucleus voxels within the data set.

Cells - Nucleus Track Area Mean
The mean of the triangle Nucleus object surfaces along the track.

Cells - Nucleus Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Cells - Nucleus Track AR1 X
TrackAR1X is the AR1 coefficient of the model for the x-positions.
For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t,t-1) - \bar{D}_x) \cdot (D_x(t+n,t+(n-1) - \bar{D}_X)$$

t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t,t-1)$$

t_L = last time index of track
t_F = first time index of track

Cells- Nucleus Track AR1 Y
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

Cells- Nucleus Track AR1 Z
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

Cells - Nucleus Track Center of Homogeneous Mass X
Cells - Nucleus Track Center of Homogeneous Mass Y
Cells - Nucleus Track Center of Homogeneous Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The voxel intensity values are taken to be the 'mass' of each voxel.
Assuming equal voxel intensity, the center of homogeneous mass is the centroid of the object. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

R : center of homogeneous mass (x y z)

r_i : center of a voxel (x_i y_i z_i)
n : number of i $\in$ Nucleus

Cells - Nucleus Track Center of Image Mass X
Cells - Nucleus Track Center of Image Mass Y
Cells - Nucleus Track Center of Image Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Image Mass is is the centroid of the object calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in object} m_i * r_i$$

R : center of image mass (x y z)
m_i : voxel intensity
r_i : center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Cells - Nucleus Track Displacement Length
The Track Displacement Length is the distance between first and last Nucleus position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_1, t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_x(t)$ = x-position of object at time index t

Cells - Nucleus Track Displacement X
Cells - Nucleus Track Displacement Y
Cells - Nucleus Track Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Cells - Nucleus Track Duration
The Track Duration is the duration between the first and last time point within the track.

Duration = TrackDuration
T(t) = time in seconds at time point t
t_L = last time index of track
t_F = first time index of track

Cells - Nucleus Track Ellipsoid Axis AX Mean
Cells - Nucleus Track Ellipsoid Axis AY Mean
Cells - Nucleus Track Ellipsoid Axis AZ Mean
These three parameters define the Vector of the track Ellipsoid Axis a.

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Cells - Nucleus Track Ellipsoid Axis BX Mean
Cells - Nucleus Track Ellipsoid Axis BY Mean
Cells - Nucleus Track Ellipsoid Axis BZ Mean
These three parameters define the Vector of the track Ellipsoid Axis b.

Cells - Nucleus Track Ellipsoid Axis CX Mean
Cells - Nucleus Track Ellipsoid Axis CY Mean
Cells - Nucleus Track Ellipsoid Axis CZ Mean
These three parameters define the Vector of the track Ellipsoid Axis c.

Cells - Nucleus Track Ellipsoid Axis Length A Mean
Cells - Nucleus Track Ellipsoid Axis Length B Mean
Cells - Nucleus Track Ellipsoid Axis Length C Mean
These three parameters define the length of the Ellipsoid Axis a, b, and c.

Cells - Nucleus Track Ellipsoid Oblate A Mean
This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

Cells - Nucleus Track Ellipsoid Prolate A Mean
This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

Cells- Nucleus Track Intensity Center Mean
This value describes the mean voxel intensity in the center of the Nucleus along the tracks.

Cells- Nucleus Track Intensity Max
This value describes the maximum voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity Mean
This value describes the mean voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity Median
This value describes the median voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity Min
This value describes the minimum voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Intensity Sum
This value describes the sum of voxel intensity of the Nucleus along the tracks.

Cells - Nucleus Track Length
The Track Length is the total length of displacements within the track.

L = TrackLength
t_L = last time index of track
t_F = first time index of track

$P_x(t)$ = x-position of object at time index t

Cells - Nucleus Track Number of Branches
The number of track branches.

Cells - Nucleus Track Number of Fusions
The number of track fusions.

Cells- Track Number of Nucleus
The number of the all Nucleus objects along the tracks.

Cells - Nucleus Tracks Position Mean X
Cells - Nucleus Tracks Position Mean Y
Cells - Nucleus Tracks Position Mean Z
Defines the mean value of X, Y, Z Nucleus track positions.

Cells - Nucleus Track Speed Max
The maximum value of the object's speed on the track.

Cells - Nucleus Track Speed Mean
The Average value of the object's speed on the track. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

Cells - Nucleus Track Speed Min
The minimum value of the object's speed on the track.

Cells - Nucleus Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Cells - Nucleus Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$\nu = \frac{\sigma}{\mu}$$

σ- Standard Deviation
μ-Mean value

Cells- Nucleus Track Sphericity Mean
Describes the mean Nucleus object Sphericity along the tracks.

Cells - Nucleus Track Straightness

S = TrackStraightness
D = TrackDisplacement
L = TrackLength

Cells - Nucleus Velocity X
Cells - Nucleus Velocity Y
Cells - Nucleus Velocity Z

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The instant velocity vector v of an object that has positions x(t) at time t and x(t + Δt) at time t + Δt, can be computed as the derivative of the position:

$$v = \lim_{\Delta t \to 0} \frac{x(t+\Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Cells - Nucleus Volume
Volume is quantification of how much space a Nucleus objects occupies.

Cells- Nucleus Track Volume Mean
The mean volume of all Nucleus objects along the tracks.

Cells - Vesicle Acceleration
Acceleration is the change in the Vesicle velocity over time.

Cells - Vesicle Acceleration X
Cells - Vesicle Acceleration Y
Cells - Vesicle Acceleration Z
Acceleration is the change in the Vesicle velocity over time along the selected-axis.

Cells- Vesicle Area
The area is the sum of the outer Vesicles voxel faces.

Cells- Vesicle Diameter
This value describes the Vesicle diameter.

Cells - Vesicles Displacement^2

$$\bar{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t,t-\Delta t)^2 + D_y(t,t-\Delta t)^2 + D_z(t,t-\Delta t)^2$$

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cells - Vesicles Displacement ^2 X
Cells - Vesicles Displacement ^2 Y
Cells - Vesicles Displacement ^2 Z
The Vesicle squared displacement along the selected-axis.

Cells- Vesicle Distance to Closest Nucleus
The distance, calculated by the distance map method, between the selected Vesicle centers and the closest Nucleus surface border.

Cells- Vesicle Distance to Closest Nucleus Center
The distance, calculated by the distance map method, between the selected Vesicle centers and the center of the closest Nucleus.

Cells- Vesicle Distance from Membrane
The distance, calculated by the distance map method, between the selected Vesicle centers and the Cell

surface border.

Cells- Vesicle Intensity Center
Cells- Vesicle Intensity Max
Cells- Vesicle Intensity Mean
Cells- Vesicle Intensity Median
Cells- Vesicle Intensity Min
Cells- Vesicle Intensity StdDev
Cells- Vesicle Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Vesicle (Center, Max, Mean, Median, Min, StdDev and Sum).

Cells - Vesicle Location in Cytoplasm
The statistical number either 0 or 1 describing the vesicle parent object. If the value is set to 1 vesicle is located within the cytoplasm, while if the value is 0 the vesicle is located within the nucleus (the value of **Vesicle location in Nucleus** is set to 1).

Cells - Vesicle Location in Nucleus
The statistical number either 0 or 1 describing the vesicle parent object. If the value is set to 1 vesicle is located within the nucleus, while if the value is 0 the vesicle is located outside the nucleus (within the cytoplasm).

Cells - Vesicle Number of Tracks
This value describes the total number of the Vesicle object tracks.

Cells - Vesicle Number of Voxels
Number of voxels within the selected Vesicle object.

Cells- Vesicle Position X
Cells- Vesicle Position Y
Cells- Vesicle Position Z
Defines the value of X, Y, Z Vesicle position (x y z).

Cells- Vesicle Relative Position
This value describes the Vesicle position within the cell relative to the Nucleus and Cell object's borders.
The value is the ratio between Vesicle distance from the Nucleus border and the sum of Vesicle's distances from the Nucleus and Cell border.

Cell - Vesicle Relative To Cell Acceleration
Acceleration is the change in the Vesicles velocity over time taking into account the relative vesicle position.

Cell - Vesicle Relative To Cell Acceleration X
Cell - Vesicle Relative To Cell Acceleration Y
Cell - Vesicle Relative To Cell Acceleration Z
Acceleration is the change in the Vesicles velocity over time along the selected-axis, taking into account the relative vesicle position.

Cells - Vesicle Number of Cell per Time Point
This value describes the number of Vesicle object in the selected time point.

Cell - Vesicle Relative To Cell Displacementˆ2
The vesicles squared displacement is calculated taking into account the relative vesicle position.

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$$D_x(t_{1,}t_2) = P_x(t_1) - P_x(t_2)$$

t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cell - Vesicle Relative To Cell Displacementˆ2 X
Cell - Vesicle Relative To Cell Displacementˆ2 Y
Cell - Vesicle Relative To Cell Displacementˆ2 Z
The vesicles squared displacement along the selected-axis, taking into account the relative vesicle position.

Cell - Vesicle Relative To Cell Position X
Cell - Vesicle Relative To Cell Position Y
Cell - Vesicle Relative To Cell Position Z
The relative Vesicles position is calculated by subtracting the absolute position of the selected Vesicles from the absolute center position of the parent cell object.

Cell - Vesicle Relative To Cell Speed
The relative speed of the vesicles is calculated taking into account the vesicle relative position.

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}}{T(t) - T(t-1)}$$

S(t) = instantaneous speed

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

PX(t) = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

$$S(0) = \sqrt{\frac{D_x(t_1 - t_0)^2 + D_y(t_1 - t_0)^2 + D_z(t_1 - t_0)^2}{T(t_1) - T(t_0)}}$$

S(0) = instantaneous speed at the first time point

Cells -Vesicle Relative To Cell Track Displacement Length
The Vesicle Relative To Cell Track Displacement Length is the distance between first and last Vesicle's relative position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_1, t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cell - Vesicle Relative To Cell Track Displacement X
Cell - Vesicle Relative To Cell Track Displacement Y
Cell - Vesicle Relative To Cell Track Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis taking into account the relative vesicle position

Cell - Vesicle Relative To Cell Velocity X
Cell - Vesicle Relative To Cell Velocity Y
Cell - Vesicle Relative To Cell Velocity Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Δt, can be computed as the derivative of the relative vesicle position along the selected-axis:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Cells - Vesicle Speed
The Vesicle speed is the instantaneous speed of the object (um/s).

$$S(t) = \frac{\sqrt{D_x(t, t-1)^2 + D_y(t, t-1)^2 + D_z(t, t-1)^2}}{T(t) - T(t-1)}$$

S(t) = instantaneous speed

$$D_x(t_1, t_2) = P_x(t_1) - P_x(t_2)$$

S(t) = TrackSpeed
PX(t) = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

S(0) = instantaneous speed at the first time point

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$$S(n)=\sqrt{\frac{D_x(t_n-t_{n-1})^2+D_y(t_n-t_{n-1})^2+D_z(t_n-t_{n-1})^2}{T(t_n)-T(t_{n-1})}}$$

$$D_x(t_n t_{n-1})=P_x(t_n)-P_x(t_{n-1})$$

S(n) = instantaneous speed at the last time point

Cells- Vesicle Time Index
The value is only available for the time series data sets. The number indicates the current time point in the series for the selected object.

Cells- Total Number of Vesicle
This value describes the total number of Vesicle objects.

Cells- Vesicles Track Area Mean
The mean area of all Vesicle objects along the tracks.

Cells- Vesicle Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Cells- Vesicle Track AR1 X
TrackAR1X is the AR1 coefficient of the model for the x-positions.
For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

$$AR1X=\frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n=\sum_{t=t_F-1}^{t_L-n}(D_x(t,t-1)-\bar{D}_x)\cdot(D_x(t+n,t+(n-1)-\bar{D}_X)$$

t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2)=P_x(t_1)-P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

t_L = last time index of track
t_F = first time index of track

Cells- Vesicle Track AR1 Y
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

Cells- Vesicle Track AR1 Z
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

Cells- Vesicles Track Diameter Mean
The mean diameter of all Vesicle objects along the tracks.

Cells- VesicleTrack Displacement Length
The Track Displacement Length is the distance between first and last Vesicle's position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_1, t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Cells- Vesicle Track Displacement X
Cells- Vesicle Track Displacement Y
Cells- Vesicle Track Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Cells - Vesicle Track Duration
The Track Duration is the duration between the first and last time point within the track.

$$\text{Duration} = \mathrm{T}(\mathrm{t}_L) - T(t_F)$$

Duration = TrackDuration
T(t) = time in seconds at time point t
t_L = last time index of track
t_F = first time index of track

Cells- Vesicle Track Intensity Center Mean
This value describes the mean voxel intensity in the center of the Vesicle along the tracks.

Cells- Vesicle Track Intensity Max
This value describes the maximum voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Mean
This value describes the mean voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Median
This value describes the median voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Min
This value describes the minimum voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Vesicle along the tracks.

Cells - Vesicle Track Intensity Sum
This value describes the sum of voxel intensity of the Vesicle along the tracks.

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Cells - Vesicle Track Length
The Track Length is the total length of displacements within the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}$$

L = TrackLength
t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

Cells - VesicleTrack Number of Branches
The number of track branches.

Cells - Vesicle Track Number of Fusions
The number of track fusions.

Cells- Vesicle Tracks Position Mean X
Cells- Vesicle Tracks Position Mean Y
Cells- Vesicle Tracks Position Mean Z
Defines the mean value of X, Y, Z Vesicles track positions.

Cells- Track Number of Vesicle
The number of the all Vesicles objects along the tracks.

Cells - Vesicle Track Speed Max
The maximum value of the object's speed on the track.

Cells - Vesicle Track Speed Mean
The Average value of the object's speed on the track. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

Cells - Vesicle Track Speed Min
The minimum value of the object's speed on the track.

Cells - Vesicle Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Cells - Vesicle Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

σ- Standard Deviation
μ-Mean value

Cells - Vesicle Track Straightness

$$S = \frac{D}{L}$$

S = TrackStraightness
D = TrackDisplacement
L = TrackLength

Cells- Vesicles Track Volume Mean
The mean volume of all Vesicle objects along the tracks.

Cells - Vesicle Velocity X
Cells - Vesicle Velocity Y
Cells - Vesicle Velocity Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Δt) at time t + Δt, can be computed as the derivative of the position:

$$v = \lim_{\Delta t \to 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Cells- Vesicle Volume
Volume is quantification of how much space a Vesicle objects occupy.

3.18.8.2 Filament

Statistic Values - Filament
In Figure 1, the presentation of the dendritic tree is simplified, by (•) representing a dendritic vertex, and (**x**) a spine vertex. Dendrite and Spine edges are represented with (—) or (····) respectively. Dendrite segments are noted with D and spine segments with S.

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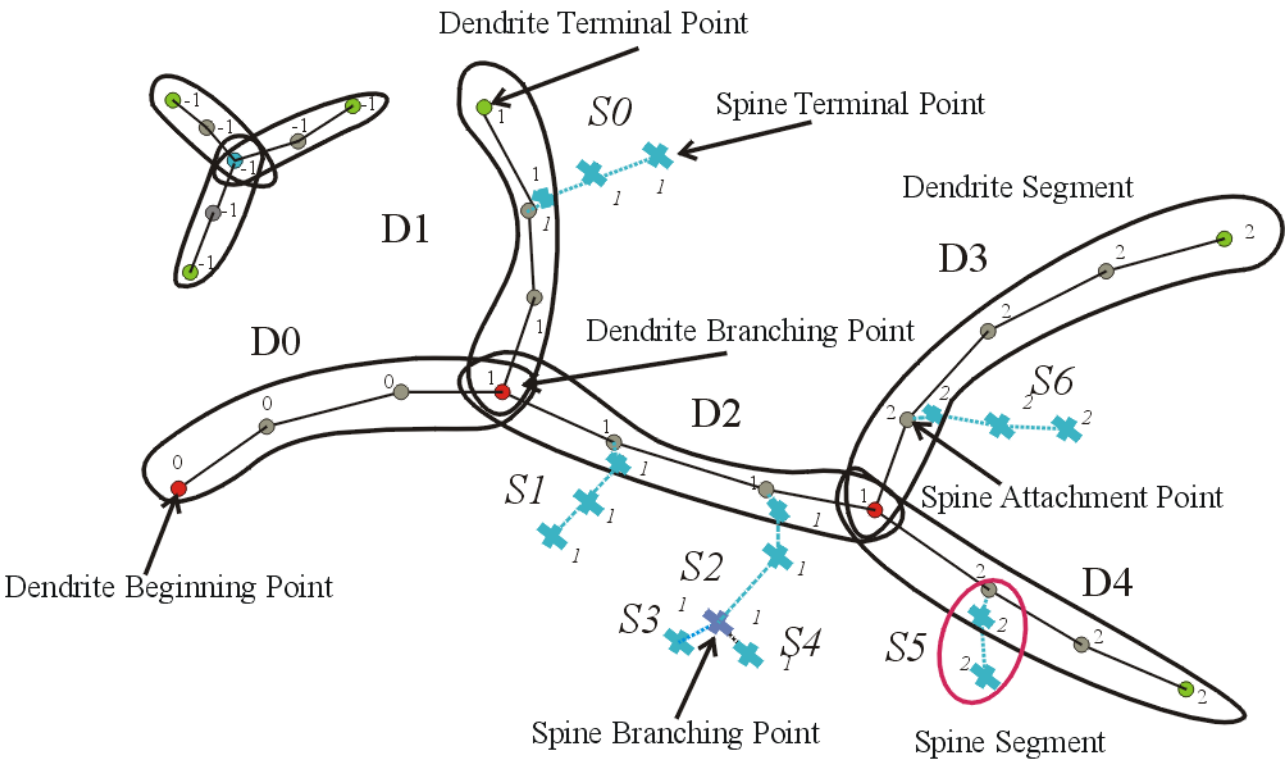

Figure 1

Filaments - Dendrite Area
Defined as the sum of the areas of all the dendrites segment edges.
The area of an edge is defined as a surface area of a frustum (truncated cone).

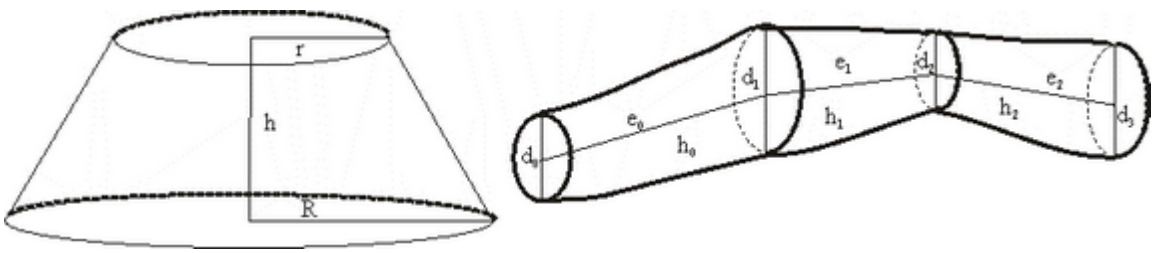

Figure 2

n =number of edges of a segment

Filaments - Dendrite Branch Depth
Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the dendritic graph.

Filaments - Dendrite Branch Level

The Dendrite Branch Level is a numerical structure that starts unfolding from the filament beginning toward the terminal points, assigning Branch Level to dendrite segments the at each branching point.
The Branch Level number is assessed according to the diameter calculations of the individual dendrite segments. The initial Branch Level at the beginning point is 1. At each branching point, the dendrite segment with smaller mean diameter sequentially increases Branch Level, while the dendrite segment with a greater diameter maintains the same Branch Level.
In the case of two dendrite segments with the same diameter, the segment with a smaller Branching Angle (refer to Figure 4) keeps the Branch Level, while the dendrite segment with a greater Branching Angle sequentially raises its Branch Level.
In the Figure 3 diameter d_1 is greater than d_2 ($d_1 > d_2$) and d_3 and d_4 are equal ($d_3 = d_4$). The Dendrite Branching Angle α_1 is smaller than α_2 ($\alpha_1 < \alpha_2$).

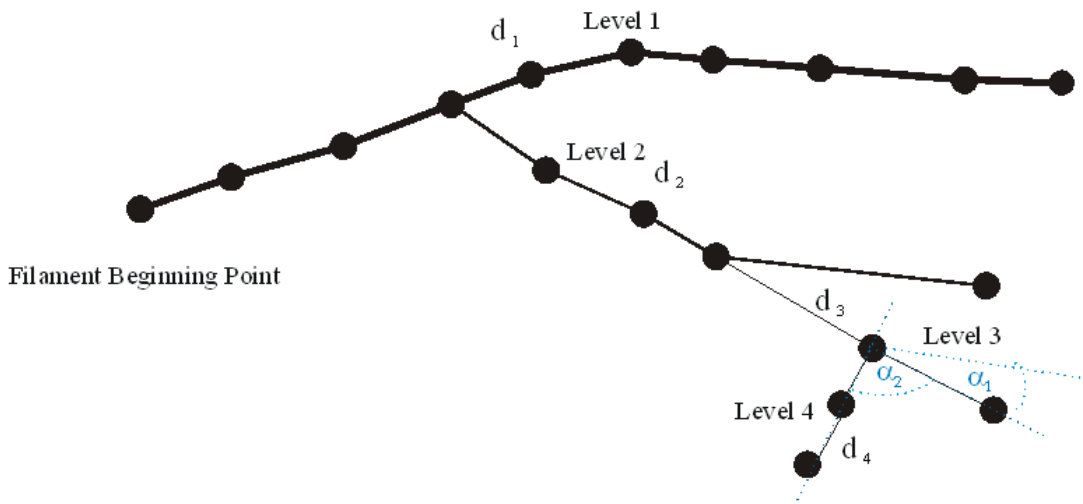

Figure 3

Filaments - Dendrite Branching Angle
Defined as the angle between the extending lines from the branch point and its peripheral neighbor (Figure 4 angles marked with dashed blue arcs, α). Defined only if beginning point is determined.

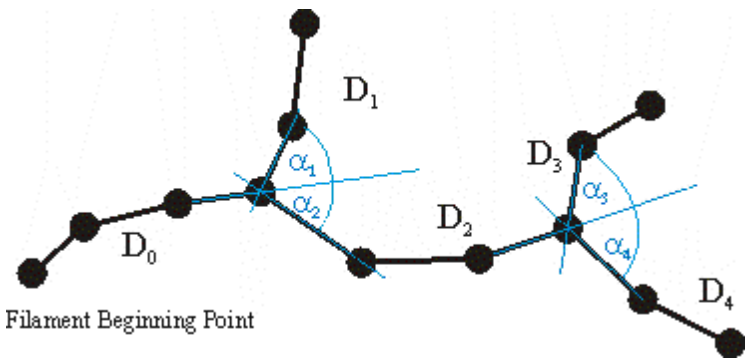

Figure 4

Filaments - Dendrite Branching Angle B
The angle between the extending lines connecting the branch point with the neighboring branch points and the terminal points, respectively (Figure 5 angles marked with dashed green arcs, β). Defined only if beginning point is determined.

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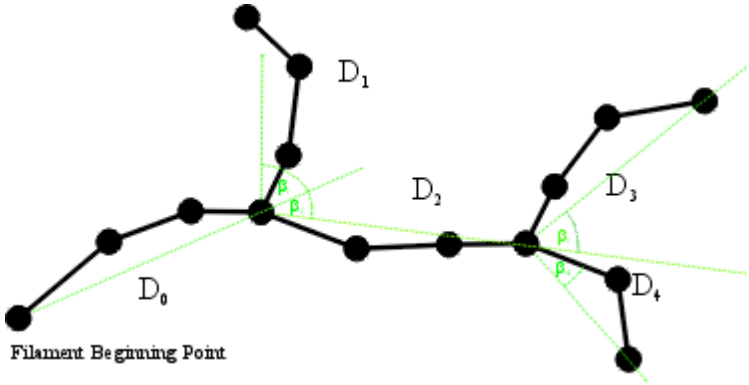

Figure 5

Filaments -Dendrite Length
Defined as the sum of all edges between two branch points or between a branch point and a terminal point, respectively. (Figure 2 Dendrite Length e0+e1+e2).

Filaments -Dendrite Mean Diameter
The mean value of all dendrites diameters measured from the center to the dendrite surfaces using the lower threshold (automatic creation) value.

Filaments -Dendrite No of Spines
The number of spines branching off a dendrite segment (in Figure1 D0 0, D11, D2 2, D3 1, D4 1)

Filaments - Dendrite Orientation Angle
The angle formed between extending line connecting distal vertices of the dendrite segment and X-axis of image within the XY plane (Figure 6 angles marked with a dashed orange arcs, γ).

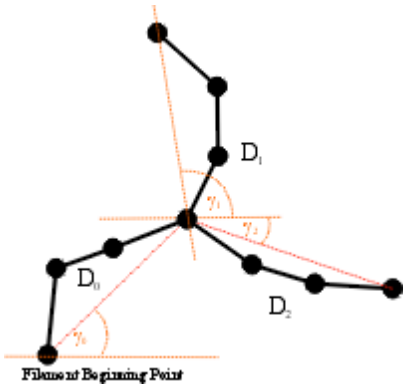

Figure 6

Filaments - Dendrite Resistance
Measure of a dendrite ability to conduct an electric current. The dendrite resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account diameter variations. It has units of 1/mm and must be multiplied by specific material resistance in order to obtain a value of a resistor.

Filaments - Dendrite Spine Density
Defined as the number of spines per unit length of dendrite.

Filaments - Dendrite Straightness
Defined as the ratio between dendrite length and radial distance between two branch points (h) (Figure 7 value is always smaller than 1. If the Dendrite Straightness is 1 that means that the dendrite is completely straight.

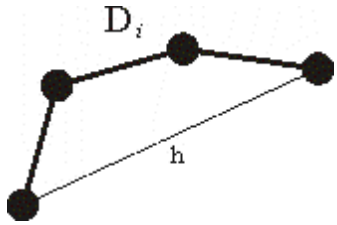

Figure 7

Filaments - Dendrite Volume
Defined as the sum of all (frustum) volumes composing a dendrite (Figure 2).

$$V(i) = \left(\frac{\pi}{3}\right) * h(i) * \left(r(i)^2 + r(i) * r(i+1) + r(i+1)^2\right)$$

$$V = \sum V(i) \quad i = 0,...n-1, n$$

n =number of edges of a segment

Filaments - Edge Diameter
Defined as the diameters measured at each vertex of the graph middle axis (Figure 2 values d_0, d_1, d_2).

Filaments - Edge Length
Defined as the length of the line connecting two vertices (Figure 2 values e_0, e_1, e_2).

Filaments - Filament Acceleration
Acceleration is the change in the filament graph velocity over time.

Filaments - Filament Dendrite Area (sum)
Defined as the sum of the areas of all dendrites segments within the graph.

Filaments -Filament Dendrite Length (sum)
The sum of all dendrites length of within the entire filament graph.

Filaments -Filament Dendrite Volume (sum)
The sum of the volumes of all dendrites within the entire filament graph.

Filaments -Filament Displacement [2]

$$\bar{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

Filaments - Filament Full Branch Depth
Branch Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the filament graph. Full Branch Depth is the highest value of Branching Depth for the graph (in Figure 1 value is 2). If no beginning point is defined, the value is set to "-1" (Figure1).

Filaments - Filament Full Branch Level
Full Branch Level is the highest value of Branching Level for the entire filament graph (in Figure 3 value is 4).

Filaments - Filament Length (Sum)
Defined as the sum of the lengths of all edges (both dendrites and spines) within the entire graph.

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Filaments - Filament No. Dendrite Branch Points
The number of dendrites branching points in the entire filament graph (in Figure1 value is 3).

Filaments - Filament No. Dendrites Branches
The number of parallel outstretched dendritic branches counted from the beginning point toward the terminal points at constant length increments. In Figure 8 the Number of Dendrite Branches are indicated with Roman numerals. The middle figure part represents overlay the branch structure onto the parallel distance intervals. The distance increments are represented with a green dashed lines and increments are calculated as they would be measured from parallel branches. The lower part illustrates the Number of Dendrite Branches in relation to distance from the beginning point.

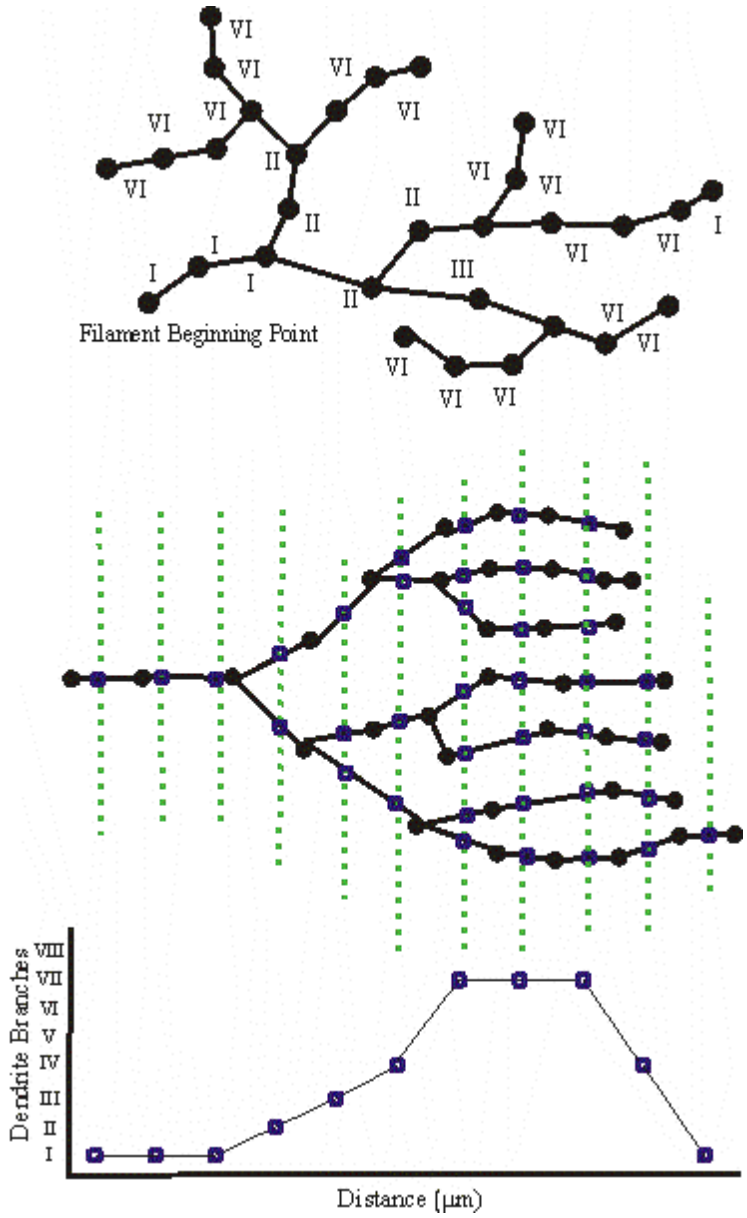

Figure 8

Filaments - Filament No. Dendrite Segments
The number of dendrite segments in the entire filament graph (Figure 1 value is 8).

Filaments - Filament No. Dendrite Terminal Points

The number of dendrite terminal points (excluding the beginning point) in the entire filament graph (Figure 1 value is 6).

Filaments - Filament No. Edges
The number of connections between vertices in the entire filament graph (Figure 1 value is 34).

Filaments - Filament No. Sholl Intersections
Defined as the number of dendrite intersections (branches) on concentric spheres, defining dendrite spatial distribution as a function of distance from the beginning point. All spheres have a center at the beginning point (Figure 9). The 1.0 um step resolution for the spheres can be changed under Preferences -> Advanced -> SurfaceAppFilament

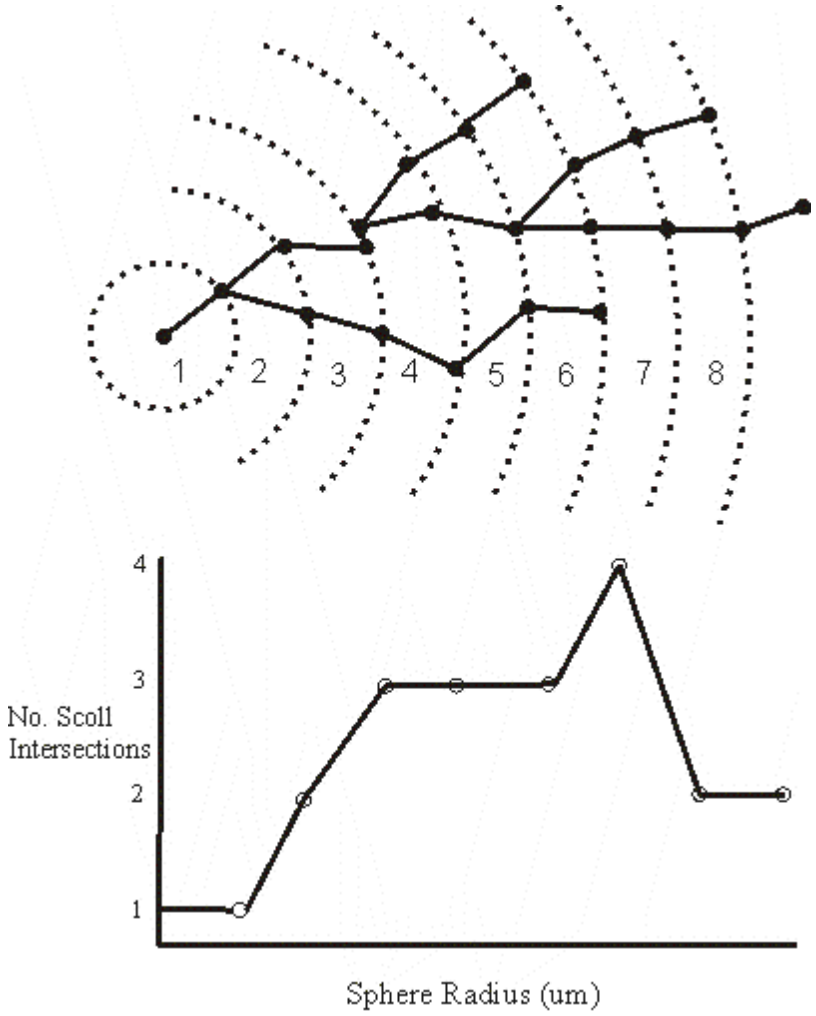

Figure 9

Filaments - Filament No. Spine Branching Points
The number of spine branch points in the entire filament graph (in Figure 1 value is 1). A spine branch point will only exist in a single spine that has more than one terminal point.

Filaments - Filament No. Spine Segments
The spine segment count in entire filament graph (in Figure 1 value is 7).

Filaments - Filament No. Spine Terminal Points
The number of spine terminal points in the entire filament graph (in Figure 1 value is 6).

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Filaments - Filament No. Unconnected Parts
The number of unconnected filament graph within the image.

Filaments - Filament No. Vertices
The number of vertices in the filament graph (Figure 1 value is 36).

Filaments – Filament Number of Filament pre Time Points
Totals number of filament graph within the image at the specific time point.

Filaments – Filament Number of Tracks
Totals number of tracks for the selected filament graph.

Filaments – Filament Position X
Filaments – Filament Position Y
Filaments – Filament Position Z
Filament graph x,y, z position within the viewing area.

Filaments – Filament Speed
The speed is the instantaneous speed of the filament graph beginning point (um/s). If beginning point is not determined the speed calculated based on the filament graph Center of Homogeneous Mass.

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}}{T(t) - T(t-1)}$$

S(t) = instantaneous speed

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

S(t) = TrackSpeed
PX(t) = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

$$S(0) = \sqrt{\frac{D_x(t_1 - t_0)^2 + D_y(t_1 - t_0)^2 + D_z(t_1 - t_0)^2}{T(t_1) - T(t_0)}}$$

S(0) = instantaneous speed at the first time point

S(n) = instantaneous speed at the last time point

Filaments - Filament Spine Area (sum)

Defined as the sum of the areas of all spines segments within the filament graph.

Filaments -Filament Spine Length (sum)
The sum of all spines length of within the entire filament graph.

Filaments -Filament Spine Volume (sum)
The sum of the volumes of all spines within the entire filament graph.

Filaments -Filament Time Index
The value is only available for the time series data sets. The number indicates the current time point in the series for the selected object.

Filaments -Filament Total Number of Filaments
The value indicates the total number of filament graph within the data set.

Filaments -Filament Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Filaments -Filament Track AR1 X
TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t,t-1) - \bar{D}_x) \cdot (D_x(t+n,t+(n-1) - \bar{D}_X)$$

t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t,t-1)$$

PX(t) = x-position of object at time index t

t_L = last time index of track

t_F = first time index of track

For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

Filaments -Filament Track AR1 Y
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

Filaments -Filament Track AR1 Z
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

Filaments -Filament Track Displacement Length
The Track Displacement Length is the distance between first and last filament graph position.

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$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_1, t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Filaments -Filament Displacement X
Filaments -Filament Displacement Y
Filaments -Filament Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Filaments -Filament Track Duration
The Track Duration is the duration between the first and last time point within the track.

$$\text{Duration} = \mathrm{T}(\mathrm{t}_L) - T(t_F)$$

Duration = TrackDuration
T(t) = time in seconds at time point t
t_L = last time index of track
t_F = first time index of track

Filaments -Filament Track Length
The Track Length is the total length of displacements along the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t, t-1)^2 + D_y(t, t-1)^2 + D_z(t, t-1)^2}$$

L = TrackLength
t_L = last time index of track
t_F = first time index of track

Filaments -Filament No. Dendrites Branches
The number of parallel outstretched dendritic branches counted from the beginning point toward the terminal points at constant length increments along the selected track.

Filaments -Filament Track Number of Fusions
A fusion occurs when a filament object is connected to several filament objects of a past time point. The value indicates the number of the related objects.

Filaments -Filament Track Number of Filaments
The total number of filament graphs along the track.

Filaments -Filament Track Position X Mean

Filaments -Filament Track Position Y Mean
Filaments -Filament Track Position Z Mean
Defines the mean value of X, Y, Z Track positions.

Filaments -Filament Track Speed Max
The maximum value of the object's speed on the track.

Filaments -Filament Track Speed Mean
The Average value of the object's speed on the track. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

Filaments -Filament Track Speed Min
The minimum value of the object's speed on the track.

Filaments - Filament Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Filaments - Filament Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$\nu = \frac{\sigma}{\mu}$$

σ- Standard Deviation
μ-Mean value

Filaments - Filament Track Straightness

$$S = \frac{D}{L}$$

S = TrackStraightness
D = TrackDisplacement
L = TrackLength

Filaments -Filament Velocity X
Filaments -Filament Velocity Y
Filaments -Filament Velocity Z
Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Δt, can be computed as the derivative of the relative object position along the selected-axis:

Filaments -Filament Volume (sum)
Volume is the quantity of three-dimensional space occupied by an object. The sum of all segment's volumes within the entire filament graph.

Filaments - Pt Acceleration
Acceleration is the change in the object velocity over time.

Filaments - Pt Branching Angle

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The angle between the lines connecting the branch point with either of its two neighboring peripheral vertices (in Figure 10 a angles are marked with δ). This value is defined only for filament graphs with a valid beginning point. If the beginning point has not been defined then Pt. Branching Angle is the smallest of the angles between the branching segments (in Figure 10 b angles marked with δ_2). The value is not calculated for branch points with more than three segments.

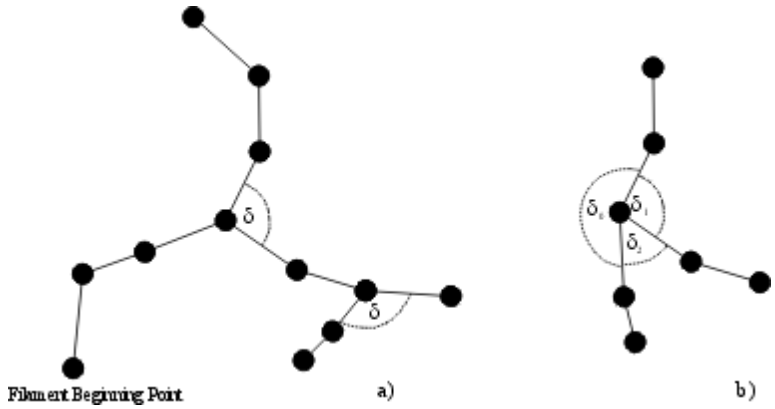

Figure 10

Filaments - Pt Diameter
Defined as the value of the vertices's diameter (Figure 2).

Filaments - Pt Displacement ^2

$$\bar{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t,t-\Delta t)^2 + D_y(t,t-\Delta t)^2 + D_z(t,t-\Delta t)^2$$

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Filaments - Pt Distance
Distance of the specific vertex from the beginning point along the dendritic graph structure (Figure 8).

Filaments - Pt Number of Points per Time Point
Number of all beginning, branching, attachment and terminal points in the selected time point.

Filaments - Pt Number of Tracks

Filaments - Pt Position X
Filaments - Pt Position Y
Filaments - Pt Position Z
The X, Y, Z coordinates of all tracked beginning, branching, attachment and terminal points.
These values are only available if the tracking option is selected.

Filaments - Pt Speed
The object speed is instantaneous speed of the graph beginning, branching, attachment and terminal points (um/s).

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}}{T(t) - T(t-1)}$$

S(t) = instantaneous speed

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

T(t) time at time point t in seconds

Filaments -Pt Time Index
The value is only available for the time series data sets. The number indicates the current time point in the series for the selected object.

Filaments -Pt Total Number of Points
Number of all beginning, branching, attachment and terminal points.

Filaments -Pt Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Filaments -Pt Track AR1 X
TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t,t-1) - \bar{D}_x) \cdot (D_x(t+n,t+(n-1) - \bar{D}_X)$$

t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

t_L = last time index of track

t_F = first time index of track

For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

Filaments -Pt Track AR1 Y
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

Filaments -Pt Track AR1 Z
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

Filaments -Pt Track Displacement Length

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The Track Displacement Length is the distance between first and last point position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_1, t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Filaments -Pt Displacement X
Filaments -Pt Displacement Y
Filaments -Pt Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Filaments -Pt Track Duration
The Track Duration is the duration between the first and last time point within the track.

$$\text{Duration} = \mathrm{T}(\mathrm{t}_L) - T(t_F)$$

Duration = TrackDuration
T(t) = time in seconds at time point t
t_L = last time index of track
t_F = first time index of track

Filaments -Pt Track Length
The Track Length is the total length of displacements along the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t, t-1)^2 + D_y(t, t-1)^2 + D_z(t, t-1)^2}$$

L = TrackLength
t_L = last time index of track
t_F = first time index of track

Filaments -Pt Track No. Dendrites Branches
The number of parallel outstretched dendritic branches counted from the beginning point toward the terminal points at constant length increments along the selected track.

Filaments -Pt Track Number of Fusions
A fusion occurs when a filament graph beginning, branching, attachment and terminal points are connected to several graph key points of a past time point. The value indicates the number of the related objects along the selected track.

Filaments -Pt Track Number of Points
The total number of points (beginning, branching, attachment and terminal) along the track.

Filaments -Pt Track Position X Mean
Filaments -Pt Track Position Y Mean
Filaments -Pt Track Position Z Mean
Defines the mean value of X, Y, Z Track positions.

Filaments -Pt Track Speed Max
The maximum value of the object's speed on the track.

Filaments - Pt Track Speed Mean
The Average value of the object's speed on the track. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

Filaments - Pt Track Speed Min
The minimum value of the object's speed on the track.

Filaments - Pt Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Filaments - Pt Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$\nu = \frac{\sigma}{\mu}$$

σ- Standard Deviation
μ-Mean value

Filaments - Pt Track Spine Area Max
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 10).
The value is maximal spine area along the track.

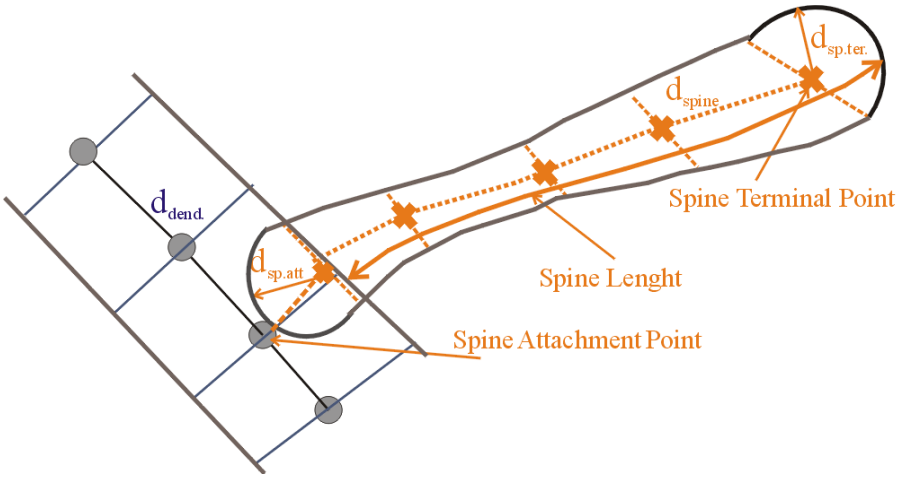

Figure 11

Filaments -Pt Track Spine Area Mean
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 11).
The value is mean spine area along the track.

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Filaments -Pt Track Spine Area Min
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 11).
The value is minimal spine area along the track.

Filaments - Pt Track Spine Length Max
The Spine length is calculated by subtracting the sphere radius at spine the attachment point (Figure 11 dsp. att) from the sum of all spine segments lengths (esp) and radius of the spine terminal point (Figure 11).
The value is maximal spine length along the track.

Filaments - Pt Track Spine Length Min
The Spine length is calculated by subtracting the sphere radius at spine the attachment point (Figure 11 dsp. att) from the sum of all spine segments lengths (esp) and radius of the spine terminal point (Figure 11).
The value is minimal spine length along the track.

Filaments - Pt Track Spine Length Mean
The Spine length is calculated by subtracting the sphere radius at spine the attachment point (Figure 11 dsp. att) from the sum of all spine segments lengths (esp) and radius of the spine terminal point (Figure 11).
The value is mean spine length along the track.

Filaments - Pt Track Spine Volume Max
The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).
The value is maximal spine volume along the track.

Filaments - Pt Track Spine Volume Min
The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).
The value is minimal spine volume along the track.

Filaments - Pt Track Spine Volume Mean
The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).
The value is mean spine volume along the track.

Filaments - Pt Track Spine Straightness
Defined as the ratio between spine length and radial distance between two branch points (h) (defined in the same way as Dendrite Straightness, refer to Figure 7).

Filaments - Pt Velocity X
Filaments - Pt Velocity Y
Filaments - Pt Velocity Z
Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Δt, can be computed as the derivative of the relative object position along the selected-axis:

Filaments - Spine Area
The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 11).

Filaments - Spine Attachments Pt Diameter
Diameter of a vertex at the spine attachment point to the dendrite (Figure 11 dsp).

Filaments - Spine Branch Depth
Spine Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the spine attachment point to a given point in the dendritic graph.

Filaments - Spine Branch Level
A Spine inherits the Dendrite Branch Level from the dendrite segment that it is attached to.

Filaments - Spine Branching
The number of spine branching points in a spine segment. The value is typically 0 or 1 (Figure 12 for S_0 0, S_1 1, S_2 0 and S_3 0).

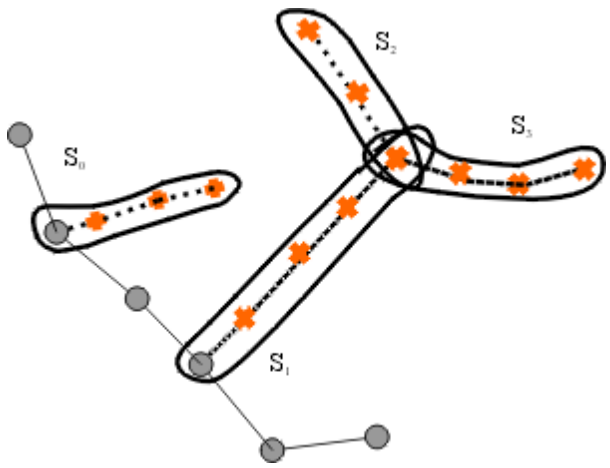

Figure 12

Filaments - Spine Length
The Spine length is calculated by subtracting the sphere radius at spine the attachment point (Figure 11 dsp. att) from the sum of all spine segments lengths (esp) and radius of the spine terminal point (Figure 11).

Filaments - Spine Max Diameter
The maximal value of spine diameters including attachment point diameter (Figure 11, average of dspine and all dsp.att).

Filaments - Spine Min Diameter
The minimal value of spine diameters including attachment point diameter (Figure 11, average of dspine and all dsp.att).

Filaments - Spine Mean Diameter
The mean value of spine diameters including attachment point diameter (Figure 11, average of dspine and all dsp.att).

Filaments - Spine Neck Max Diameter
Filaments - Spine Neck Mean Diameter
Filaments - Spine Neck Min Diameter
Maximum, Mean and Minimum value of the spine neck diameter (excluding the spine attachment and terminal pints diameters).

Filaments - Spine Neck Volume
The Spine Neck volume is calculated by subtracting the volume of the spine-ending hemispheres (attachment and terminal point) from the spine volume.

Filaments - Spine Orientation Angle

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The angle formed between the extending line connecting distal spine vertices in the spine segment and X-axis of the image within the XY plane (defined in the same way as Dendrite Orientation Angle, refer to Figure 6).

Filaments - Spine Resistance
Measure of a spine's ability to conduct an electric current. The spine's resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account the diameter variations. It has units of 1/um and must be multiplied by specific material resistance in order to obtain a value of resistor (defined in the same way as Dendrite Resistance).

Filaments - Spine Straightness
Defined as the ratio between spine length and radial distance between two branch points (h) (defined in the same way as Dendrite Straightness, refer to Figure 7).

Filaments - Spine Terminal Point Diameter
Diameter of a vertex at the spine terminal point (Figure 11 dsp.ter.)

Filaments - Spine Terminal Pt Intensity Center
Defined as the intensity of the spine terminal center voxel.

Filaments -Spine Terminal Pt. Volume
Spine Terminal Pt. Volume is calculated as a volume of the sphere with the diameter of a spine terminal point vertex (Figure 11).

Filaments - Spine Volume
The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).

See also:
Surpass View - Filament - Tab Statistics

3.18.8.3 Measurement Points

Measurement Point statistics are automatically computed for each Measurement Point.

Show Statistic Values - Measurement Points

Measurement Points - Distance
Distance between the measurement points.

Measurement Points - Distance Sum
Sum of all measured distances.

Measurement Points - Number of Points
Number of measurement points.

Measurement Points - Point Intensity Center
Value of the point intensity center (average) in the different channels.

Measurement Points - Point Position X
Point position in x-axis.

Measurement Points - Point Position Y
Point position in y-axis.

Measurement Points - Point Position Z
Point position in z-axis.

See also:
Surpass View - Measurement Points - Tab Statistics

3.18.8.4 Spots

Spots statistics are automatically computed for each Spots object. To each Spot belongs a spatial position along the x-, y-, and z-axis, as well as the intensity of the point it represents.

Show Statistic Values - Spots

Spots- Acceleration
Acceleration is the change in the Spot(s) velocity over time.

Spots - Area
The sum of the triangle surfaces.

Spots - Diameter
The spots diameter.

Spots - Displacement ^2

$$\bar{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t,t-\Delta t)^2 + D_y(t,t-\Delta t)^2 + D_z(t,t-\Delta t)^2$$

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Spots - Intensity Center
Intensity of the voxel in the Spot(s) center.

Spots - Intensity Max
Spots - Intensity Mean
Spots - Intensity Median
Spots - Intensity Min
Spots - Intensity StdDev
Spots - Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Spots (Max, Mean, Median, Min, StdDev and Sum).

Spots - Number of Points per Time Point
Number of points per time point.

Spots - Number of Tracks
Number of tracks.

Spots - Number of Voxels
Number of voxels within the selected Spot objects.

Spots - Position X
Spots - Position Y
Spots - Position Z
Spots position (x y z).

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Spots - Speed
The spots speed is the instantaneous speed of the object (um/s).

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}}{T(t) - T(t-1)}$$

S(t) = instantaneous speed

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

S(t) = TrackSpeed
$P_x(t)$ = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

$$S(0) = \sqrt{\frac{D_x(t_1 - t_0)^2 + D_y(t_1 - t_0)^2 + D_z(t_1 - t_0)^2}{T(t_1) - T(t_0)}}$$

$$D_x(t_1 t_0) = P_x(t_1) - P_x(t_0)$$

S(0) = instantaneous speed at the first time point

$$S(n) = \sqrt{\frac{D_x(t_n - t_{n-1})^2 + D_y(t_n - t_{n-1})^2 + D_z(t_n - t_{n-1})^2}{T(t_n) - T(t_{n-1})}}$$

$$D_x(t_n t_{n-1}) = P_x(t_n) - P_x(t_{n-1})$$

S(n) = instantaneous speed at the last time point

Spots - Time Index
The value is only available for the time series data sets. The number indicates the current time point in the series for the selected object.

Spots - Total Number of Spots
The total number of spots objects.

Spots - Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Spots - Track AR1 X
TrackAR1X is the AR1 coefficient of the model for the x-positions.
For more information, please visit: http://en.wikipedia.org/wiki/Autoregressive_model

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t,t-1) - \bar{D}_x) \cdot (D_x(t+n,t+(n-1) - \bar{D}_X)$$

t_L = last time index of track
t_F = first time index of track

$$D_x(t_{1,}t_2) = P_x(t_1) - P_x(t_2)$$

$P_X(t)$ = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t,t-1)$$

t_L = last time index of track
t_F = first time index of track

Spots - Track AR1 Y
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

Spots - Track AR1 Z
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

Spots - Track Area Mean
The mean of the triangle Spots surfaces along the track.

Spots - Track Diameter Mean
The mean diameter of all spots along the track.

Spots - Track Displacement Length
The Track Displacement Length is the distance between first and last spot's position.

$$D_x(t_n,t_{n-1}) = \sqrt{D_x(t_L,t_F)^2 + D_y(t_L,t_F)^2 + D_z(t_L,t_F)^2}$$

$$D_x(t_1,t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Spots-Track Displacement X
Spots-Track Displacement Y
Spots-Track Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Spots - Track Duration
The Track Duration is the duration between the first and last time point within the track.

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Duration = TrackDuration
T(t) = time in seconds at time point t
t_L = last time index of track
t_F = first time index of track

Spots - Track Intensity Center Mean
The mean center intensity of all spots along the track.

Spots - Track Intensity Max
The max intensity of all spots along the track.

Spots - Track Intensity Mean
The mean intensity of all spots along the track.

Spots - Track Intensity Median
The median track intensity of all spots along the track.

Spots - Track Intensity Min
The min track intensity of all spots along the track.

Spots - Track Intensity StdDev
The standard deviation track intensity of all spots along the track.

Spots - Track Intensity Sum
The sum of the track intensity of all spots along the track.

Spots - Track Length
The Track Length is the total length of displacements within the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}$$

L = TrackLength
t_L = last time index of track
t_F = first time index of track

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

$P_x(t)$ = x-position of object at time index t

Spots - Track Number of Branches
The number of track branches.

Spots - Track Number of Fusions
The number of track fusions.

Spots - Track Number of Spots
The number of track Spots.

Spots - Tracks Position Mean X
Spots - Tracks Position Mean Y
Spots - Tracks Position Mean Z
Defines the mean value of X, Y, Z track coordinates positions.

Spots - Track Speed Max
The maximum value of the object's speed on the track.

Spots - Track Speed Mean
The Average value of the object's speed on the track. If the Track has no merges or splits, then the average speed is given by the Track length divided by the time between first and last object in the Track.

Spots - Track Speed Min
The minimum value of the object's speed on the track.

Spots - Track Speed Standard Deviation
The Standard Deviation value of the object's speed on the track.

Spots -Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$\nu = \frac{\sigma}{\mu}$$

σ- Standard Deviation
μ-Mean value

Spots - Track Straightness

$$S = \frac{D}{L}$$

S = TrackStraightness
D = TrackDisplacement
L = TrackLength

Spots - Track Volume Mean
The mean volume of all Spots along the tracks.

Spots - Velocity X
Spots - Velocity Y
Spots - Velocity Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Δt) at time t + Δt, can be computed as the derivative of the position:

Spots - Volume
Volume is a quantification of how much space an Spots object occupies.

See also:
Addendum - Terminology (Standard Deviation)

3.18.8.5 Surfaces

Surfaces statistics are automatically computed for each Surface.

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Show Statistic Values - Surfaces

Surfaces - Acceleration
Acceleration is the change in the Surface velocity over time.

Surfaces - Area
The sum of the triangle surfaces.

Surfaces - Center of Homogeneous Mass X
Surfaces - Center of Homogeneous Mass Y
Surfaces - Center of Homogeneous Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The voxel intensity values are taken to be the 'mass' of each voxel.
Assuming equal voxel intensity, the center of homogeneous mass is the centroid of the object. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in object} r_i$$

R : center of homogeneous mass (x y z)
r_i : center of a voxel (x_i y_i z_i)
n : number of i $\in$ Surfaces

Surfaces - Center of Image Mass X
Surfaces - Center of Image Mass Y
Surfaces - Center of Image Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Image Mass is is the centroid of the object calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in object} m_i * r_i$$

R : center of image mass (x y z)
m_i : voxel intensity
r_i : center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Surfaces - Displacement ^2

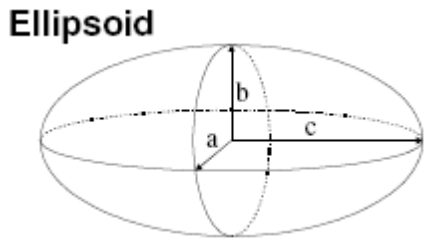
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Ellipsoid Axis

In mathematics, an Ellipsoid is a type of quadric that is a higher dimensional analogue of an Ellipse.
The equation of a standard Ellipsoid in an x-y-z Cartesian coordinate system is:

$$\frac{x^2}{a^2}+\frac{y^2}{b^2}+\frac{z^2}{c^2}=1$$

where a, b and c (the lengths of the three semi-axes) are fixed positive real numbers determining the shape of the Ellipsoid.



Fitting ellipsoid into the surface

To calculate the dimension of a three principal axes of the best-fitted ellipsoid into the surface, the moments of inertia along the axes of ellipsoid is computed and optimized to match the moment of inertia of the surface. The ellipsoid has homogeneous mass distribution within the surface object. For more information:

http://en.wikipedia.org/wiki/Moment_of_inertia#Principal_moments_of_inertia

Please note: Only if the shape of the surface matches to an ellipsoid, the lengths of the three ellipsoid axes are exactly equal to the surface axis.

Surfaces - Ellipsoid Axis A X
Surfaces - Ellipsoid Axis A Y
Surfaces - Ellipsoid Axis A Z
These three parameters define the Vector of the Ellipsoid Axis a.

Surfaces - Ellipsoid Axis B X
Surfaces - Ellipsoid Axis B Y
Surfaces - Ellipsoid Axis B Z
These three parameters define the Vector of the Ellipsoid Axis b.

Surfaces - Ellipsoid Axis C X
Surfaces - Ellipsoid Axis C Y
Surfaces - Ellipsoid Axis C Z
These three parameters define the Vector of the Ellipsoid Axis c.

Surfaces - Ellipsoid Axis Length A
Surfaces - Ellipsoid Axis Length B
Surfaces - Ellipsoid Axis Length C
Surfaces - Ellipsoid (oblate)
Surfaces - Ellipsoid (prolate)

If we assume $a \leq b \leq c$, then when:

- a = 0 it is an **Ellipse**
- a = b = c it is a **Sphere** (three equal sides)

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- $a \neq b \neq c$ it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

- a = b < c it is a prolate Spheroid (cigar-shaped)
- a < b = c it is an oblate Spheroid (disk-shaped)

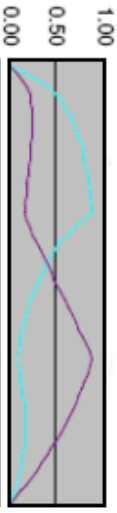
$$e_{prolate} = \frac{2a^2}{a^2+b^2} \cdot \left(1 - \frac{a^2+b^2}{2c^2}\right)$$

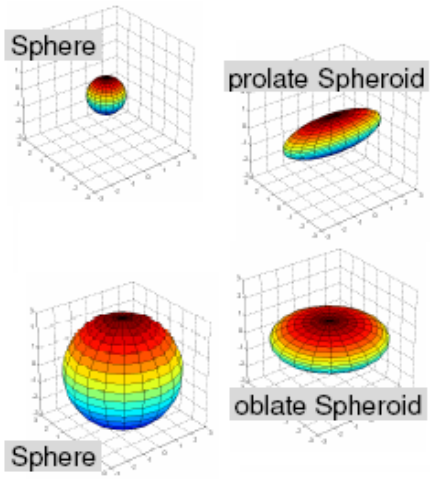
$e_{prolate}$ = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2+c^2} \cdot \left(1 - \frac{2a^2}{b^2+c^2}\right)$$

e_{oblate} = oblate Ellipsoid

a	b	c	ep	eo	Shape
1	1	1	**0.00**	**0.00**	Sphere
1	1	1.5	0.56	0.24	prolate Spheroid
1	1	2.0	0.75	0.24	prolate Spheroid
1	1	2.5	0.84	0.20	prolate Spheroid
1	1	3	**0.89**	0.16	prolate Spheroid
1	1.5	3	0.50	0.33	prolate Ellipsoid
1	2.0	3	0.29	0.52	Ellipsoid
1	2.5	3	0.16	0.71	oblate Ellipsoid
1	3	3	0.09	**0.89**	oblate Spheroid
1.5	3	3	0.15	0.75	oblate Spheroid
2.0	3	3	0.17	0.56	oblate Spheroid
2.5	3	3	0.13	0.31	oblate Spheroid
3	3	3	**0.00**	**0.00**	Sphere





Surfaces - Intensity Center
Surfaces - Intensity Max
Surfaces - Intensity Mean
Surfaces - Intensity Median
Surfaces - Intensity Min
Surfaces - Intensity StdDev
Surfaces - Intensity Sum
The voxel intensity statistics describe the voxels enclosed within the Surfaces (Center, Max, Mean, Median, Min, StdDev and Sum).

Surface- Number of Disconnected Components
The number of disconnected surfaces in the selected Surface object .

Surface- Number of Disconnected Components per time point
The number of created disconnected surfaces in the selected time point.

Surfaces - Number of Surfaces per Time Point
Number of Surfaces per actual Time Point.

Surfaces - Number of Tracks
Number of tracks.

Surfaces - Number of Triangles
Number of the Surface object triangles.

Surfaces - Number of Vertices
Number of the Surface object vertices.

Surfaces - Number of Voxels
Number of voxels within the selected Surface object.

Surfaces- Position X,Y,Z
The X, Y and Z coordinates of the surface object.

Surfaces - Speed
The Track Speed is the instantaneous speed of the object.

$$S(t) = \frac{\sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}}{T(t) - T(t-1)}$$

S(t) = instantaneous speed

$$D_x(t_1,t_2) = P_x(t_1) - P_x(t_2)$$

S(t) = TrackSpeed
$P_x(t)$ = x-position of object at time index t
T(t) = time in seconds at time point t

For the first and the last time points in the series the instantaneous speed calculation of based on the formula:

$$S(0) = \sqrt{\frac{D_x(t_1 - t_0)^2 + D_y(t_1 - t_0)^2 + D_z(t_1 - t_0)^2}{T(t_1) - T(t_0)}}$$

$$D_x(t_1 t_0) = P_x(t_1) - P_x(t_0)$$

S(0) = instantaneous speed at the first time point

$$S(n) = \sqrt{\frac{D_x(t_n - t_{n-1})^2 + D_y(t_n - t_{n-1})^2 + D_z(t_n - t_{n-1})^2}{T(t_n) - T(t_{n-1})}}$$

S(n) = instantaneous speed at the last time point

Surface- Sphericity
Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity, Ψ, of a particle is the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of the particle:

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$$\Psi = \frac{\pi^{1/3}(6V_p)^{2/3}}{A_P}$$

Vp = volume of the particle
Ap = surface area of the particle

Surfaces - Time Index
The value is only available for the time series data sets. The number indicates the current time point in the series for the selected object.

Surface- Total Number of Disconnected Components
Total number of created disconnected surfaces.

Surfaces - Total Number of Surfaces
Total number of Surfaces.

Surfaces - Total Number of Triangles
Total number of Triangles.

Surfaces - Total Number of Voxels
Total number of voxels of the Surface objects within the dataset.

Surfaces - Velocity X
Surfaces - Velocity Y
Surfaces - Velocity Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Δt) at time t + Δt, can be computed as the derivative of the position:

$$v = \lim_{\Delta t \to 0} \frac{x(t+\Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

Surfaces - Track AR1Mean
TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Surfaces - Track AR1 X
TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

t_L = last time index of track
t_F = first time index of track

$P_X(t)$ = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t, t-1)$$

t_L = last time index of track
t_F = first time index of track

Surfaces - Track AR1 Y
TrackAR1Y is the AR1 coefficient of the model for the y-positions (see above for x-position).

Surfaces- Track AR1 Z
TrackAR1Z is the AR1 coefficient of the model for the z-positions (see above for x-position).

Surfaces - Track Area Mean
The mean of the triangle surfaces along the track.

Surfaces - Track Center of Homogeneous Mass X
Surfaces - Track Center of Homogeneous Mass Y
Surfaces - Track Center of Homogeneous Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The voxel intensity values are taken to be the 'mass' of each voxel.
Assuming equal voxel intensity, the center of homogeneous mass is the centroid of the object. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in object} r_i$$

R : center of homogeneous mass (x y z)
r_i : center of a voxel (x_i y_i z_i)
n : number of i $\in$Surfaces

Surfaces - Track Center of Image Mass X
Surfaces - Track Center of Image Mass Y
Surfaces - Track Center of Image Mass Z
The voxel intensity values are taken to be the 'mass' of each voxel.
The Center of Image Mass is is the centroid of the object calculated by taking into account the voxel intensities of each channel separately.
The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in object} m_i * r_i$$

R : center of image mass (x y z)
m_i : voxel intensity
r_i : center of a voxel (x_i y_i z_i)
M : sum of voxel intensities

Surfaces - Track Displacement Length
The Track Displacement Length is the distance between first and last surface's position.

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$$D_x(t_1, t_2) = P_n(t_n) - P_n(t_{n-1})$$

D = TrackDisplacement
t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Surfaces -Track Displacement X
Surfaces -Track Displacement Y
Surfaces -Track Displacement Z
The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

Surfaces - Track Displacement^2

$$\bar{D}^2(\Delta t) = \sum_{t-t_r+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_x(t_1, t_2) = P_x(t_1) - P_x(t_2)$$

t_L = last time index of track
t_F = first time index of track
$P_X(t)$ = x-position of object at time index t

Surfaces - Track Duration
The Track Duration is the duration between the first and last time point within the Track.

$$\text{Duration} = \mathrm{T}(\mathrm{t}_L) - T(t_F)$$

Duration = TrackDuration
T(t) = time in seconds at time point t
t_L = last time index of track
t_F = first time index of track

Surfaces - Track Ellipsoid Axis AX Mean
Surfaces - Track Ellipsoid Axis AY Mean
Surfaces - Track Ellipsoid Axis AZ Mean
These three parameters define the Vector of the track Ellipsoid Axis a.

Surfaces - Track Ellipsoid Axis BX Mean
Surfaces - Track Ellipsoid Axis BY Mean
Surfaces - Track Ellipsoid Axis BZ Mean
These three parameters define the Vector of the track Ellipsoid Axis b.

Surfaces - Track Ellipsoid Axis CX Mean
Surfaces - Track Ellipsoid Axis CY Mean
Surfaces - Track Ellipsoid Axis CZ Mean
These three parameters define the Vector of the track Ellipsoid Axis c.

Surfaces - Track Ellipsoid Axis Length A Mean
Surfaces - Track Ellipsoid Axis Length B Mean
Surfaces - Track Ellipsoid Axis Length C Mean
These three parameters define the length of the Ellipsoid Axis a, b, and c.

Surfaces - Track Ellipsoid Oblate A Mean
This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

Surfaces - Track Ellipsoid Prolate A Mean
This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

Surfaces - Track Intensity Center Mean
This value describes the mean voxel intensity in the center of the Surfaces.

Surfaces - Track Position X Mean
Surfaces - Track Position Y Mean
Surfaces - Track Position Z Mean
Defines the mean value of X, Y, Z Track positions

Surfaces - Track Intensity Max
This value describes the maximum voxel intensity of the Surfaces along the tracks.

Surfaces - Track Intensity Mean
This value describes the mean voxel intensity of the Surfaces along the tracks.

Surfaces - Track Intensity Median
This value describes the median voxel intensity of the Surfaces along the tracks.

Surfaces - Track Intensity Min
This value describes the minimum voxel intensity of the Surfaces along the tracks.

Surfaces - Track Intensity StdDev
This value describes the Standard Deviation voxel intensity of the Surfaces along the tracks.

Surfaces - Track Intensity Sum
This value describes the sum of voxel intensity of the Surfaces along the tracks.

Surfaces - Track Length
The Track Length is the total length of displacements within the Track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}$$

L = TrackLength
t_L = last time index of track
t_F = first time index of track

$P_X(t)$ = x-position of object at time index t

Surfaces - Track Number of Branches
A branch occurs when a Surface object is connected to several other Surface objects of a future time point.

Surfaces - Track Number of Fusions
A fusion occurs when a Surface object is connected to several Surface objects of a past time point.

Surfaces - Track Number of Surfaces
The total number of Surfaces along the track.

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Surfaces - Track Number of Triangles
Number of Triangles along the track.

Surfaces - Track Number of Voxels
Number of Voxels along the track.

Surfaces - Track Position X Mean
Surfaces - Track Position Y Mean
Surfaces - Track Position Z Mean
Defines the mean value of X, Y, Z track coordinates positions.

Surfaces - Track Speed Max, Mean, Min, StdDev
Maximum, mean, minimum and standard deviation of the value of the object's speed on the track.

Surfaces - Track Speed Variation
The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value.The coefficient of variation is a dimensionless number.

$$\nu = \frac{\sigma}{\mu}$$

σ- Standard Deviation
μ-Mean value

Surfaces - Track Sphericity Mean
Describes the mean Surfaces Sphericity along the tracks.

Surfaces - Track Straightness

$$S = \frac{D}{L}$$

S = TrackStraightness
D = TrackDisplacement
L = TrackLength

Surfaces - Velocity X
Surfaces - Velocity Y
Surfaces - Velocity Z
The instant velocity vector v of an object that has positions x(t) at time t and x(t + Δt) at time t + Δt, can be computed as the derivative of the position:

Surfaces - Track Volume Mean
The mean volume of all Surfaces along the tracks.

Surfaces - Volume
Volume is a quantification of how much space a Surface object occupies.

See also:
Surpass View - Surfaces - Tab Statistics

3.18.8.6 Volume

Volume statistics are automatically computed for each Volume object.

Show Statistic Values - Volume

Volume - Data Intensity Mean
Volume - Data Intensity StdDev
Volume - Data Intensity Sum
The voxel intensity statistics describe the voxels of the whole dataset (Mean, StdDev and Sum).

Volume - Data Volume
Volume is a quantification of how much space an object occupies.

Volume - Number of Voxels
Number of the Volume voxels.

See also:
Surpass View - Volume - Tab Statistics
Addendum - Terminology (Standard Deviation)

3.18.9 Custom Tools

Fiji Application

Browse
Here you can specify the Fiji folder. Either type in the respective path or use the button **Browse**.

Matlab

Please note: The specification of the path to Matlab is only necessary for the Mac users

The following examples show the location of the MATLAB application.

Matlab Application: /Applications/MATLAB_R2007b/bin/matlab
Matlab runtime environment: /Applications/MATLAB/MATLAB_Compiler_Runtime/v711

Button **Browse**
Click on this button to browse for the special folder.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.18.10 Licenses

Overview

Node-locked License
Node-locked licenses grant permission to run the licensed software on one computer (node) only. Node-locked licenses are the easiest way to license the software. You can use the License Administrator to directly download a node-locked license from the Bitplane server. If your computer is not connected to the Internet it is also possible to download this license from another computer and then copy it to the target machine. Please refer to the offline license method for further instructions.

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Floating License
Floating licenses grant permission to run the licensed software on several computers. One floating license can activate only one instance of the program at any given time. Floating licenses require the installation of a separate license server that runs on a Windows computer. The server installation requires more technical know how.
The License Administrator application easily let you enable a floating license.

Demo License
A Demo License is a time-limited (usually one month) license. It is not an official licensing scheme and therefore can't be purchased. It's main purpose is for internal Testing and to grant customers with licensing issues an easy and quick workaround so that their productivity can continue until the problem is solved. Simply open the License Administrator application and open the Demo License you received from your local support representative.

Evaluation License
Evaluation Licenses are temporary licenses allowing customers to explore all fascinating possibilities Imaris offers before actually purchasing it. The license is only limited in time (10 days), but not in features. Additionally an Evaluation License is issued per user.
To get an Evaluation License you will have to register yourself first at:

http://www.bitplane.com/go/download/imaris-evaluation

An automatic mail response should provide you with your personal RegistrationID and its corresponding Passcode. These credentials can then be entered during the application's start up.

See also:
Preferences Dialog
Installation of Licenses
Administrative Privileges
License Information
Add License

3.18.10.1 Preferences Dialog

Here you can adjust the default license settings.
License Administrator is an application designed to manage the licenses of various Bitplane applications. It provides a simple interface to add or delete any licenses. When a license is added, the license file gets copied to a system folder where it is secure from (accidental) manipulation by standard users. Although it is possible for administrators to modify license files directly in this folder we strongly advise you not to do so. It is recommended to use the License Administrator application instead.

License Administrator

This button starts the License Administrator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use the License Administrator to set up your licenses.

Please note that you'll need administrative privileges in order to start the License Administrator.

Any change of licenses will require a new start of Imaris.

Except the Evaluation License which is bound to a single user, all other licenses are bound to the whole system. Therefore any license modifications need administrative privileges.

If you have access to an administrative system account, the License Administrator should just start normally.

Start License Administrator Button

This button starts the License Administrator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use the License Administrator to set up your licenses. Please note that you'll need administrative privileges in order to start the License Administrator.
Any change of licenses will require a new start of Imaris.

Evaluation License

After you entered your RegistrationID and Passcode you can click on the Light Bulb button to activate the Evaluation License.
This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the light bulb will turn on .
You can also disable your Evaluation License by clicking again on Light Bulb button and the light bulb will turn off. This indicates that you aren't using any Evaluation Licenses.
Any change of licenses will require a new start of Imaris.

RegistrationID
Please enter your RegistrationID you received by mail first.

Passcode
Please enter your Passcode you received by mail first.

Available Licenses

The table displays all existing Imaris licenses on the system and additional information for each feature:

Version
The latest Imaris version this license can be used with.
Type
Which type of licensing scheme is used. An overview is given in the Licenses section.
Status
Displays the status of a license.
Expiration
Shows the number of days until this license expires.

License colors
In order to gain a quick overview, the validity of a license is also indicated by different colors:
– Green: License is valid.
– Yellow: License is valid, but all licenses are currently in use.
– Red: License has expired.
– No color: License is not available (e.g. this additional license feature hasn't been purchased).

You can select with which license(s) your Imaris should start per default. Any change will require a new start of Imaris.

Check/Uncheck All

Check this box to enable or disable all available Imaris licenses on the system.

Please note that the Feature “ImarisBase” can't be disabled because this would render Imaris unusable.

3.18.10.2 Installation of Licenses

First-time Installation

If you have installed Imaris for the very first time, you have to set up a license before you can use Imaris.

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A dialog window appears during the start up of the application.

The same dialog window appears if you have accidentally deleted the license .

You now have two options how to proceed:

• If you have a Node-locked or a Floating License you should use the License Administrator application for the further license setup. This is done by simply clicking on the “Start License Administrator” button. Please note that the License Administrator application needs administrative privileges.

• If you want to evaluate Imaris first, please register yourself first at:
http://www.bitplane.com/go/download/imaris-evaluation

The automatic mail response contains your RegistrationID and Passcode.
To enable an Evaluation License enter these values in the corresponding fields and click on the “Use Evaluation License” button.
Please note that a working internet connection is needed to start up Imaris in Evaluation mode.

Installation of additional Licenses

If you already have set up a license but you want to add other licenses as well, you can do this in the License Preferences dialog.

Once more, you'll have the option either to start the License Administrator application or to use an Evaluation License in addition if you would like to try out a new feature before buying the license.

3.18.10.3 License Administrator

This chapter contains information related to licensing management, options and procedures. It includes the following sections:

Administrative Privileges
License Information
Add License

3.18.10.3.1 Administrative Privileges

There are some cases where the proper privileges can not be provided automatically and requires you to start the application manually.

Windows XP:
If you are logged in with a limited user account you need to start the application with a right click and then select “Run As...” to gain access to an administrator account. Of course, if you log yourself in with an administrator account you can avoid this and the License Administrator should just start normally.

Windows Vista:
If the User Account Control (UAC) is disabled and you are logged in with a standard user no elevation prompt is shown because the UAC setting omits these prompts. Therefore you can't get the right administrative permissions to start the License Administrator application. You will either have to log in as an administrator or re-enable the User Account Control.

Windows 7:
If the User Account Control (UAC) is disabled and you are logged in with a standard user no elevation prompt is shown because the UAC setting omits these prompts. Therefore you can't get the right administrative permissions and you will have to start the License Administrator manually. This is done by opening the executable by a right click while holding down the “Shift” key. Then you can select “Run as different User” which prompts you for another account.

Of course, the Windows Vista workarounds do still apply: you can enable UAC again or log in as administrator directly.

Mac OS X:
No cases known where problems occur.

Default License Administrator Paths

Windows XP, Windows Vista and Windows 7
32-bit: C:\Program Files (x86)\Bitplane\Imaris 7.3.0\LicenseAdministrator.exe
64-bit: C:\Program Files\Bitplane\Imaris 7.3.0\LicenseAdministrator.exe

Mac OS X
/Applications/Imaris 7.3.0 beta3.app/Contents/MacOS/LicenseAdministrator

3.18.10.3.2 Show License Information Window

If you did not have set up a license yet (e.g. first-time usage) this window is empty. In that case, please use the **Add License Location** button to add a new license location.

Add License Location Button
Use this button to add new licenses. A description is given in the Add license Window chapter.

Delete License Location
If you click this button, the selected license location entry in the table underneath will be deleted. Before using this button, please make sure you selected the right table entry. In case of a node-locked license you are asked if you want to keep the license. It is recommended that you keep all license files.

License Location Table
This table lists all available license locations. Normally it would be just enough to have one license location defined. But there exist also good reasons to use multiple locations.

For example you might have a floating license server with just some basic features enabled. Maybe later you purchased some additional features in a node-locked licensing scheme. You can now just define both locations and the licensed features are merged on your system automatically.

The table shows also some additional Information:
- Type: This is either a License Server (floating license scheme) or a License File (all other schemes)
- Number of valid / total Licenses: These numbers shows you how many different features your license contains and it lets you easily verify that all licenses are still valid.

If you click on another table entry, the License table underneath gets updated and shows you detailed information about all available features of the current selected license location.

Licenses Table
In this table you have a detailed overview of all licensed features of a license location. You can switch among different license locations by selecting the corresponding entry in the License Location table.

Each feature provides some information :
Version
The latest Imaris version this license can be used with.
Type
Which type of licensing scheme is used. An overview is given in the Licenses section.
Status
Displays the status of a license.
Expiration
Shows the number of days until this license expires.

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License colors
In order to gain a quick overview, the validity of a license is also indicated by different colors:
– Green: License is valid.
– Yellow: License is valid, but all licenses are currently in use.
– Red: License has expired.
– No color: License is not available (e.g. this additional license feature hasn't been purchased).

Finish Button
This button is only enabled if you added at least one license location. It is an indicator when all necessary steps to set up a license are done. If all features in the above License table are highlighted in green (or only some of them) you successfully managed to add a license. If you click the button, it just closes the License Administrator and you can proceed using Imaris.

3.18.10.3.3 Add License Window

First, you have to select your licensing scheme:

Download Node-locked License

If you decide to add a Node-locked License, it is checked if your computer is connected to the internet first. If the connection could be established successfully, the Online-Licensing Wizard is shown; if not, you have the possibility to download the license from another computer which is described in the Offline-Licensing Wizard.

Online-Licensing Wizard
This wizard has three steps:

1. Select the Network Interface
You need to select the network interface which is bound to the license. If your Host-ID is already known to our server, the available product for installation will be shown in the table below.

2. Check your Order-ID for newly ordered Products:
If you have ordered new products, you have to enter the Order-ID in this step. Clicking on the “Check Order-ID” button will add the products to the table below. Once you checked an Order-ID, it is bound to the currently selected network interface.
If your Host-ID is already known to the system, the available products will be shown in the tables below and you could skip that step.

3. Select the Licenses for Installation
In this step you just have to mark all products you want to install by checking them in the left table.
The right table is for your information only and shows all currently installed products.
When you click on the “OK” button, the license file is downloaded and the corresponding entry is added to the License Location table.

Offline-Licensing Wizard
If you are not connected to the Internet, this could have two reasons: it could be that:
1. your computer is behind a web proxy server which omits a direct connection or
2. you could be intentionally unplugged .

Therefore you have the following choices:

A) Set up a Proxy Server
Enter your proxy server's name and its port and click on the **Test Connection** button. If the connection could be established you are automatically redirected to the Online-Licensing Wizard described above.
If not, either the credentials you entered are not correct or the connection is cut at another place in the network. In that case please contact your network administrator or Bitplane Support teams eusupport@bitplane.com or ussupport@bitplane.com.

If you want to delete a previously set up proxy server, just delete both fields and click on the button again.
The proxy server settings can also be altered in Imaris' system preferences dialog.

B) Download the Node-locked License from another Computer
You can download the license from another computer connected to the internet.
On that machine log on to http://www.bitplane.com/activation/acti_offline_ident.html
and enter the Host-ID you wish to bind to the license together with your Order-ID into the web form. The download of the license should immediately start and you could transfer the file afterwards to the offline computer (e.g. by using a USB stick).
This instructions can be saved in a file by clicking the **Save Instructions...** button.

When you have downloaded the license you can just open it with the **Open License...** button or enter its path directly into the field on the left of the button.
When you click **OK** the license file is copied to the proper location.

Define Floating License Server

In order to add a Floating License Server, just enter the server's name and its port to the corresponding fields and click on the **OK** button. The default port is 2700.

Install License from Existing File

If you received a Demo License from your local support representative or you need to add a license file directly you can use this wizard. If you click on the **Open License...** button you can browse to the license you want to add. You can also specify the license file to load by entering its path directly into the field left from the button. If you click on the **OK** button, the license is copied to the right location and it's added to your License Location table.

3.18.11 Update

Here you can adjust the default update settings.

How often should be searched for updates?

Daily
Weekly
Never
Select either daily, weekly, or never.

Button OK
To apply the changes click on **OK**.

Button Cancel
If you do not want to save the changes click on **Cancel**.

3.18.12 Usage Data

Here you can enable the capturing of data usage .

Button OK
To apply the changes click on **OK**.

Button Cancel
If you do not want to save the changes click on **Cancel**.

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3.18.13 3D Cursor

Here you can adjust the default 3D Cursor settings.

3D Cursor

Box
Sphere
Cross
Torus
Here you can select the shape of the 3D Cursor.

Size
Color
Here you can select the size and color of the 3D Cursor.

Animation

This are the default animation parameters for the function InMotion.

Frequency
Adjust the animation frequency.

Amplitude X
Amplitude Y
Adjust the amplitude in x-, and y-direction.

Shear
Rotate
Select shear or rotate as animation mode.

Continuous
Discrete
Select continuous or discrete as animation sequence.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

See also:
InMotion

3.18.14 InPress

Here you can adjust the default InPress settings.

Format

Title Format
Text Format
Legend Format
Choose title format, text format or legend format and adjust the following parameter set.

Title, Text or Legend

Font
Font Size
Color
Format
Bold
Italic
Underlined
The font, font size, color, and format can be selected (from any installed system font).

Button **Select...**
Click on this button to open the color selection window to change the respective color.

Style

Arrow Color
Arrow Width
Arrow Style
Text Box Color
Text Box Transparency
Text Box Style
You can adjust the arrow color, width and style (Sphere, Arrow, Magnifier), text box color, transparency and style (Unicolor, Black Text, Black Title).

Button **Select...**
Click on this button to open the color selection window to change the respective color.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.18.15 Advanced

Displays a window similar to the parameters window. It shows internal settings and parameters in groups and parameter lists.

Please keep in mind: Changing the advanced parameter settings may endanger your system and lead to erroneous shutdowns!

Button **Reset Registry**
Click on this button reset registry values to their default settings.

Button **OK**
To apply the changes click on **OK**.

Button **Cancel**
If you do not want to save the changes click on **Cancel**.

3.19 File Type Associations...

The file association dialog for the thumbnail creator (window Imaris Icon and Thumbnail Settings) is displayed.

File Extensions

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Thumbnails will be created and MetaData will be extracted from all checked file types.

Please note: The feature of the thumbnailextractor is not available for the Macintosh users.

.ims
Imaris Series, Imaris Classic

.imx
Imaris Scene

.ics .ids
Nikon ICS

.lsm
Zeiss LSM510

.zvi
Zeiss Axiovision

.lei
Leica LCS

.lif
Leica LIF

.pic
Biorad MRC 1024/600

.rbinf
TILLvisION

.stk
MetaMorph STK

.r3d .dv
Delta Vision

.oib .oif
Olympus OIB/OIF

.ipl
Scanalytics IPLab

.ome
Open Microscopy Environment XML

.inf .info
Leica Series

.tif .tiff
Zeiss LSM 410, Zeiss LSM 310, Leica TCS-NT, Leica Series, Leica LCS, Olympus FluoView, Olympus cellR, PerkinElmer, TIFF series

More
Separated by spaces: e.g. ".ims .ics .lsm .zvi".

2D/3D Indicator

The generated thumbnails are created with or without a 2D/3D indicator.

Show
Check this box to show the indicator at the left upper corner of the thumbnail. There are four different icons to indicate the image type:

- 2D Image (colored square).
- 2D Time Image (pink square and bars).
- 3D Image (green cube).
- 3D Time Image (blue cube and bars).

4 Menu View

		Please refer to chapter:
Slice	Ctrl + 1	Slice View
Section	Ctrl + 2	Section View
Gallery	Ctrl + 3	Gallery View
Easy 3D	Ctrl + 4	Easy 3D View
Surpass	Ctrl + 5	Surpass View
Animation	Ctrl + 6	Animation
InMotion	Ctrl + 7	InMotion
Coloc	Ctrl + 8	Coloc
Fit	Ctrl + B	
100%	Ctrl + A	
Full Screen	F11	
Navi	Ctrl + N	
Origin Bottom Left		
Origin Top Left		
Origin Bottom Right		
Origin Top Right		
Status Bar		Toolbars - Status Bar
Refresh	Ctrl + F	

See also:
Addendum - Mouse & Keyboard Mac

4.1 Fit

Fits the entire scene into the view.

In the Status Bar you find the respective button **Fit**.

See also:
Toolbars - Status Bar

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4.2 100%

Select 100% to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.
In the Status Bar you find the respective button **100%**.

See also:
Toolbars - Status Bar

4.3 Full Screen

Displays the viewing area to full size of the monitor. To return to the standard window, click on the button **Full Screen** in the lower left corner.

In the Status Bar you find the respective button **Full Screen**.

See also:
Toolbars - Status Bar

4.4 Navi

The Navigation window displays an overview of the data set in the upper right corner of the viewing area. If you move the data set the overview updates in real-time. You can activate the Navigation window individually for each Imaris view.

In the Status Bar you find the respective button **Navi**.

Change Position

- In the pointer mode Select you can click & drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section view the position of the Navigation window is fixed (lower right corner) and can not be moved.

See also:
Section View
Toolbars - Status Bar
Surpass View - Overview - Camera Toolbar (Pointer Select)

4.5 Origin

The location of the coordinate system origin can be set to any corner of all the views. It can be set to: Bottom Left (default), Top Left, Bottom Right, or Top Right.A selection is stored and used each time you start Imaris.

4.6 Refresh

Refresh the screen.

5 Menu Image Processing

The Image Processing menu provides a range of functions to change the image data. Frequently used functions are Baseline Subtraction, and Threshold Cutoff from the Image Thresholding submenu or the Gaussian Filter from the Image Smoothing submenu.

Button Apply
If a time series is loaded, clicking **Apply** applies the image processing function to the current time point displayed on the screen.

Button OK
Clicking **OK** applies the image processing function to all time points.

Flip ...
Rotate...
Channel Shift...
Swap Time and Z
Swap Time and Channels

Smoothing
- Gaussian Filter...
- Median Filter...

Thresholding
- Baseline Subtraction...
- Threshold Cutoff...
- Background Subtraction...
- Connective Baseline...

Contrast Range
- Linear Stretch...
- Gamma Correction...
- Invert...
- Normalize Layers...
- Histogram Equalization

AutoDeblur...
XTensions

5.1 Flip...

The channels can be flipped together or separately.

On the left hand side of the window Flip Channel(s) the actual channel(s) are displayed. Select the channels to be flipped by marking the check-box on the left.

All Channel(s)

Direction X, Y, Z
Check the requested Direction box.

Button Apply
Button OK
Button Cancel
Click the **Apply** button. The effect of the change can be seen in the image. If necessary, change the direction.

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When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

5.2 Rotate...

Rotate direction applies to all channels together. Single channel application is not possible.

All Channel(s)

Axis X, Y, Z
Enter the requested axis.

Orientation
Counter Clockwise, Clockwise
Select the respective orientation.

Button **Apply**
Button **OK**
Button **Cancel**
Click the **Apply** button. The effect result can be seen in the image. If necessary, change the direction. When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

5.3 Free Rotate

The Free Rotation function rotates an image by any degree and offers additional image resizing options. The camera location determines the rotation angles and directions. The center of the image is the center point of the rotation. The operation is preformed for every time point and channel.
Please note selecting just one channel for free rotation will create two data sets.

X, Y, Z

These fields display the current x-, y-, and z-values.
To alter the image position you can write the values directly into the fields or you can as well adjust the image position in the Surpass View.

Angle

This field displays the current value of the angle of rotation.

Coordinate resize factor

The image and voxel values define the size of the new data set after free rotation.
These values are used to build a map of all the co-ordinates that will be used to display the newly rotated image.

Free rotation can be an expensive memory operation. Adjusting the image dimensions can reduce the size of

the data set, decreasing the time needed to read the file and speeding up calculation. This can be particularly important when reading large data sets.

Voxel

Changing the x, y, and z values creates a new image with different voxel spacing.
These values determine the resampling points. Resampling reduces or increases the voxel density in a data set.
Reducing the resampling points deteriorates the image but speed up any operations on the data.

X, Y, Z
In the Voxel X, Y and Z fields, enter the factor by which to multiply the current resampling interval.
For decimation (fewer samples), enter a factor greater than 1 to increase the sampling interval by this factor.
For interpolation (more samples), enter a factor less than 1 to decrease the sampling interval by this factor.

Image

If the border of the new image needs to be adjusted, change the x, y, and z values and create a new image with the different image size. This option helps you to crop (minimize the size) the dataset without loosing significant data.

X, Y, Z
The parameters X, Y and Z allow you to specify the size of a rotated image by defining the range of an original image.
In the Image X, Y and Z fields, enter the crop factor of the lower and upper image range.
The values of X, Y and Z should be in the range between 0 and 1.

Padding Intensity

When you create a new image with free rotation some of the new voxel surroundings are included with the dataset. The padding voxels define an area between the previous outside boundary of the original image and newly rotated image content.
This padding intensity field lets you edit the newly created pad voxel intensity.

Linear interpolation

After rotation and data size adjustment, a new image with different voxel spacing is created and the new voxel coordinates need to be calculated.
The difference between the voxel spacing on the new and old image means that an interpolation scheme is required as the new image's voxels are unlikely to coincide with those in the old image.
The Linear interpolation box allows you to select the interpolation method.
If the box is selected for new voxel coordinates then the tri-linear interpolation method is used (all surrounding values are used for calculations).
If the box is not selected the voxel spacing will be calculated based on the nearest neighbor interpolation
Depending on the dataset and parameter values the interpolation algorithm could involve some rather time intensive internal calculations. When speed is favored, please consider selecting the less-time costly interpolation method (nearest-neighbor).

5.4 Channel Shift...

The Channel Shift function allows to move channels relative to one another.

On the left hand side of the window Channel Shift the actual channel(s) are displayed. Select the channels that should be moved relative to the remaining channels.

Pixel Shift for All Selected Channel(s)

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X, Y, Z
The translation is specified in terms of integer numbers of voxels to move along each of the x-, y-, and z-directions.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

5.5 Swap Time and Z

Exchange the t-axis and the z-axis and re-format the image in such a way that the time dimension is mapped onto the z-axis and the z-dimension is mapped onto the t-axis. The section viewer now shows x-t-, and y-t-views.

Start Function

- Select in the menu Image Processing - Swap Time and Z.

The z-, and t-axes are exchanged. This allows you to play the images over z and t.

Play the Series

- Click on Start (arrow to the right) in the Time Bar to play the series of images along the z-axis.

The Time Bar is visible in all display modes (Slice, Section, Gallery 3D Preview, and Surpass). It allows you to view a particular Time Point or to automatically play 4D images. The views change when selecting a new Time Point.

Example:
Your data set has four Time Points, each Time Point has one Slice in z-dimension. Then you swap time and z. The result is a new data set with one Time Point and four Slices in z-dimension.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

See also:
Toolbars - Time Bar

5.6 Swap Time and Channels

Exchange the t-axis and the channels and re-format the image.

Start Function

- Select in the menu Image Processing - Swap Time and Channels.

The t-axes and the channels are exchanged.

Example:
Your data set has four Time Points, each Time Point has one channel. Then you swap time and channel. The result is a new data set with one Time Point and four channels.

Button **Add to Batch**

Add to Batch button places the command into Batch coordinator.

See also:
Menu Edit - Image Properties - Channel 1 ... n
Menu Edit - Show Display Adjustment

5.7 Extract Channel

The Extract Channels compare the color content of an image to reference color information. This function extracts and displays color data from the image referent point and use it as a representative color, for a new color channel creation. The new channel is generated based on the difference in the reference color point and the whole image color values.
The difference relates how closely the color information in the region matches the information represented by the color reference point, assigning the higher intensity for smaller difference.The result of the Extract Channel is a separate channel, which can be processed and viewed as any originally acquired channel in Imaris and used for easier segmentation of similarly colored multi-channel data sets.

- Click on the **Slice** icon in the Main toolbar.
- Change pointer mode from Navigate to **Select**.
- Select the reference color and click on it.
- Click on the Get value from the last measurement point Button displays associated RGB values in the numerical fields.
- Specify Color Difference by selecting the color space (either RGB or Perceptual).
- Select the Apply button. The effect of the change can be seen on the screen.
- When the results are satisfactory, click the OK button.
- The new channel is created and it's a part of the data set.
- To restore the initial image click Cancel.

The new extracted channel can be visualized like any other channel in Imaris by selecting the Edit menu and choosing the option **Display Adjustment Window**. In the Display Adjustment Window uncheck original channels, select the newly created channel and adjust its setting.

Channels
The channels can be selected by clicking on the check box.

Color of Interest
The Color of Interest option allows the reference color point to be present either as separate value for red green and blue or as the intensity value for a selected channel.
The reference color point is shown within the color wheel.

RGB
RGB triple value is displayed for the color reference point.

Intensity
Intensity of the selected channel is displayed for the color reference point.
Get value from the last measurement point Button
Clicking this button calculates the RGB or intensity values according to the selected color reference point.

Color difference
This option defines the color space of a newly created channel.

RGB
The RGB color space is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors.

Perceptual

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Implements the color extraction using Lab color space. The Lab color model provides a perceptually uniform color space. A perceptually uniform color space indicates that a change in a color value produces a change of about the same visual importance. Lab color is designed to approximate human vision.
For more specific information about Lab color space: http://en.wikipedia.org/wiki/Lab_color_space

5.8 Smoothing

Whether or not images need to be smoothed depends on their quality. Inspect the image to see if any smoothing is required. For image smoothing several different filters are available:

- Gaussian Filter
- Median Filter

5.8.1 Gaussian Filter

User Interface

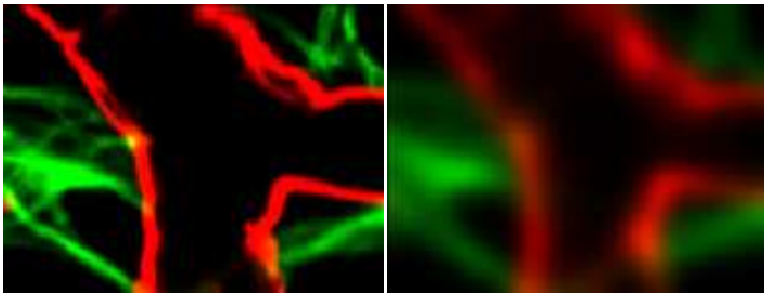
The Gaussian filter for a data set can have only one value. This value can be applied separately to any one channel or to all channels. Structures smaller than the filter width will be removed.

- Select the menu Image Processing - Image Smoothing - Gaussian Filter. The Gaussian Filter box is displayed.
- Select the channels to be filtered by marking the check-box on the left of the channel.
- Enter the requested filter width in the parameter field and click **Apply**. The effect of the change can be seen on the screen.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

The data set is modified. To restore the initial image click **Cancel**.

Function

The Gaussian filter smoothes the image.



It has some nice properties:

- Total image intensity is preserved.
- Noise is suppressed homogeneously.
- No artefacts are introduced.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

See also:
Surpass View - Filament - Data Input and Filtering

5.8.2 Median Filter

User Interface

The Median filter for a data set can have only one value. This value can be applied separately to any one channel or to all channels. Structures that are smaller than the filter width will be removed.

- Select the menu Image Processing - Image Smoothing - Median Filter. The Median Filter box is displayed.
- Select the channels to be filtered by marking the check-box.
- Check the requested Filter Size and click the **Apply** button. The effect of the change can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

The data set is modified. Click **Cancel** to return to the original image.

Function

The Median Filter replaces the intensity of each voxel with the statistical median of the intensities of neighboring voxels. There are several choices for the neighboring voxels to be included in the median calculation. The median filter is useful when the image data are corrupted by salt and pepper noise.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

5.9 Thresholding

Whether or not it is necessary to apply a thresholding function to an image depends on its quality. Inspect the image to see if any action is required.

For image thresholding a number of functions are available. The following paragraphs describe the user interface to the following filters:

- Threshold Cutoff
- Baseline Subtraction
- Background Subtraction
- Connective Baseline

5.9.1 Threshold Cutoff

Threshold Cutoff compares the intensity of every voxel in the image to the threshold value. If the intensity is greater than the threshold, it remains unchanged. If a voxels has an intensity less than the threshold value it is assigned a new intensity value. This value is 0 by default. The Threshold Cutoff values for the channels may differ.

- Select the menu Image Processing - Image Thresholding - Threshold Cutoff. The Threshold Cutoff box is displayed, showing a histogram of the voxels in the data set.
- Select the channels to be cut by marking the check-box to the left of the channel bar.
- Highlight a channel.
- A colored bar in the histogram shows which channel has been selected.
- Enter the requested values for that channel in the Threshold field or drag the bar in the histogram.

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- Repeat for each channel (if applicable).
- Click **Apply**. The effect of the changes can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

The data set is modified. Click **Cancel** to return to the original image.

5.9.2 Baseline Subtraction

Baseline Subtraction subtracts the baseline value from the intensity of every voxel in the image. If the result is positive, this is the new intensity value, if it is negative, the voxels intensity is set to zero. The Baseline Subtraction values for the channels may differ.

- Select the menu Image Processing - Image Thresholding - Baseline Subtraction. The Baseline Subtraction box is displayed, showing a histogram of the voxels in the data set.
- Select the channels to be cut by marking the check-box to the left of the channel bar.
- Highlight a channel.
- A colored bar in the histogram shows which channel has been selected.
- Enter the requested value for that channel in the Baseline field or drag the bar in the histogram.
- Repeat for each channel (if applicable).
- Click **Apply**. The effect of the changes can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

The data set is modified. Click **Cancel** to return to the original image.

5.9.3 Background Subtraction

Background Subtraction applies a Gaussian filter to define the background at each voxel and then performs a Baseline Subtraction of this variable background. The filter width is the only parameter of the background subtraction method.

- Select the menu Image Processing - Thresholding - Background Subtraction. The Background Subtraction box is displayed.
- Select the channels by marking the check-box on the left of the channel.
- Enter the requested filter width in the parameter field and click **Apply**. The effect of the change can be seen on the screen.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

5.9.4 Connective Baseline

The Connective Baseline works with two threshold values, a lower and an upper threshold. It applies these thresholds to divide the image into regions and then processes each region with a Baseline Subtraction using

either the lower or the upper threshold. The three types of regions the method creates are the following:

- Connected region of voxels where the intensity of every voxel within the region is below the lower threshold (Type A).
- Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold and no voxel within the region has an intensity greater than the upper threshold (Type B).
- Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold and at least one voxel within the region has an intensity greater than the upper threshold (Type C).

The intensities of voxels from regions of type A are set to zero, as are the intensities of voxels from regions of type B. Only voxels from regions of type C retain a nonzero intensity, namely the original intensity less the lower threshold value.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

See also:
Surpass View - Filament - Data Input

5.10 Contrast Change

For image Contrast Change a number of functions are available.
Linear Stretch...
Gamma Correction...
Invert...
Normalize Layer(s)
Histogram Equalization

5.10.1 Linear Stretch...

Linear stretching may be necessary whenever 12 bit data is read in the 16 bit mode. Then the contrast can be extended to the new limits. Picture data is changed when stretching. Linear stretching results in a picture displaying dense data in a wider contrast range.

- Select the menu Image Processing - Contrast Change - Linear Stretch. The Linear Stretch box is displayed.
- Select the channels to be stretched by marking the check-box.
- Enter the requested values in the parameter fields New Maximum and New Minimum and click the **Apply** button. The effect of the change can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

The data set is modified. Click **Cancel** to return to the original image.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

5.10.2 Gamma Correction...

The Gamma correction function lets you improve the image display by intensifying the gray value of a specific range of voxels. Usually the color contrast values of the voxels stretch linear over a wide range (e.g. 0 - 255). The Gamma correction function lets you set a new gamma value according to which the gamma curve for the voxels is calculated automatically. Thus the brightness of the voxels can be intensified or weakened in certain areas.

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- Select the menu Image Processing - Contrast Change - Gamma Correction. The Gamma Correction box is displayed with the default Gamma Value set to 1, therefore showing a straight line as a linear voxel representation.
- Select the requested channels by clicking marking the check-box.
- Enter the requested value in the Gamma value field or click directly in the gray Gamma Curve area to set the new gamma value point. The curve and the field value are set accordingly and the effect of the change can be seen on the channels (channels appear differently).
- Click the **Apply** button
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

The data set is modified. Click **Cancel** to return to the original image

5.10.3 Invert...

The channels can be inverted together or separately.

- Select the menu Image Processing - Contrast Change - Invert. The Invert box is displayed.
- Select the channels to be inverted by marking the check-box on the left. The effect of the change can be seen in the image.
- When the results are satisfactory, click the **OK** button.

The data set is modified.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

Click **Cancel** to return to the original data set.

5.10.4 Normalize Layer(s)

This normalization adjusts brightness and contrast of individual z-slices to a uniform level. It is most useful for correcting illumination variations of scans from physical sections, and in some cases it can also reduce attenuation effects from z-stacks.
This image processing function first computes the mean and standard deviation values of the intensity for an entire image z-stack. Then it computes mean and Std.dev. for all individual z-slices. Finally, it adjusts the intensities of each z-slice with a linear transformation, to match the overall mean and Std.dev. value.
This procedure is applied for all z-slices of the dataset, for all time points and for each channel independently.
Voxels with zero intensity are excluded from this calculation - they are interpreted as background.

- Select the menu Image Processing - Contrast Change - Normalize.
- Select the channels to be inverted by marking the check-box on the left and click on the button **Apply**.
- When the results are satisfactory, click the **OK** button.

The data set is modified.

Button **Add to Batch**
Add to Batch button places the command into Batch coordinator.

Click **Cancel** to return to the original data set.

5.10.5 Histogram Equalization

Histogram equalization is a method of image processing by contrast adjustment using the image's histogram. The method is useful in images with close contrast values; in particular, this method can lead to better views of electron microscopy images.

Through this adjustment, the dynamic range and contrast of an image is altered. The resulting image features the highest possible contrast, uniformly distributed over the whole contrast range.
This method increases the global contrast by adjusting the distribution of the intensities on the histogram by spreading out the most frequent values. In histogram equalization, each pixel is assigned a new intensity value based on its previous intensity level so that the output image contains a uniform distribution of intensities. This allows for areas of lower local contrast to gain a higher contrast without affecting the global contrast.

Current minimum/maximum
These values define the limits of dataset intensities. Intensities below the Current minimum will be set to the new minimum and intensities above the Current maximum will be set to the new maximum.
New minimum/maximum
These values define the intensity range of the output dataset. By default, they are set to the minimum/ maximum achievable intensities of the data set.

To perform histogram equalization on the data set perform the following steps:

- Select the menu Image Processing - Contrast Change - Histogram Equalization.
- The Equalize Channel Histogram(s) window is displayed
- Select the channels to be processed by marking the check-box
- Enter the requested values in the parameter fields Current Minimum/Maximum, New Maximum/Minimum or accept the automatically optimized Min. and Max. limits.
- Click the **Apply** button.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

Add to Batch Button
Add to Batch button places the command into Batch coordinator.

Click **Cancel** to return to the original data set.

5.11 Auto Deblur ...

The AutoDeblur deconvolution software is integrated into Imaris. It requires a separate license. The pathway to AutoDeblur must be set in the menu Edit - Preferences... - Advanced - Files - AutoDeblurExeFileName.

When opening AutoDeblur, the software will prompt for a destination for the file that is transferred to AutoDeblur and will automatically create an image in the ids/ics format and open it in AutoDeblur.

- Select the menu Image Processing - AutoDeblur ....

An error message appears if the program is not installed or the pathway is not set.

5.12 XTensions

Enter topic text here.

6 Fiji

Imaris Fiji compatible plugins are aimed at performing additional image processing and offering a solution for specific problems with image analysis.
The Imaris Fiji menu allows you to use your current Fiji routines and still continue with your work within the Imaris. The Fiji plugins are integrated into Imaris and they can be executed from and within the application, enhancing and extending the functions of Imaris.
In the Fiji menu you can find a list of plugins organized into a menu structure. Each plugin collection is

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displayed as a sub menu on this menu. The plugins and macros are stored and run from the Fiji folder.

Image From Fiji
Image To Fiji
File
Image
Process
Analyze
Plugins
Options…
Help..

Please note: This is not an Fiji tutorial. These chapters describe only the usage of Fiji as an integrated part of Imaris.

6.1 Fiji-Imaris

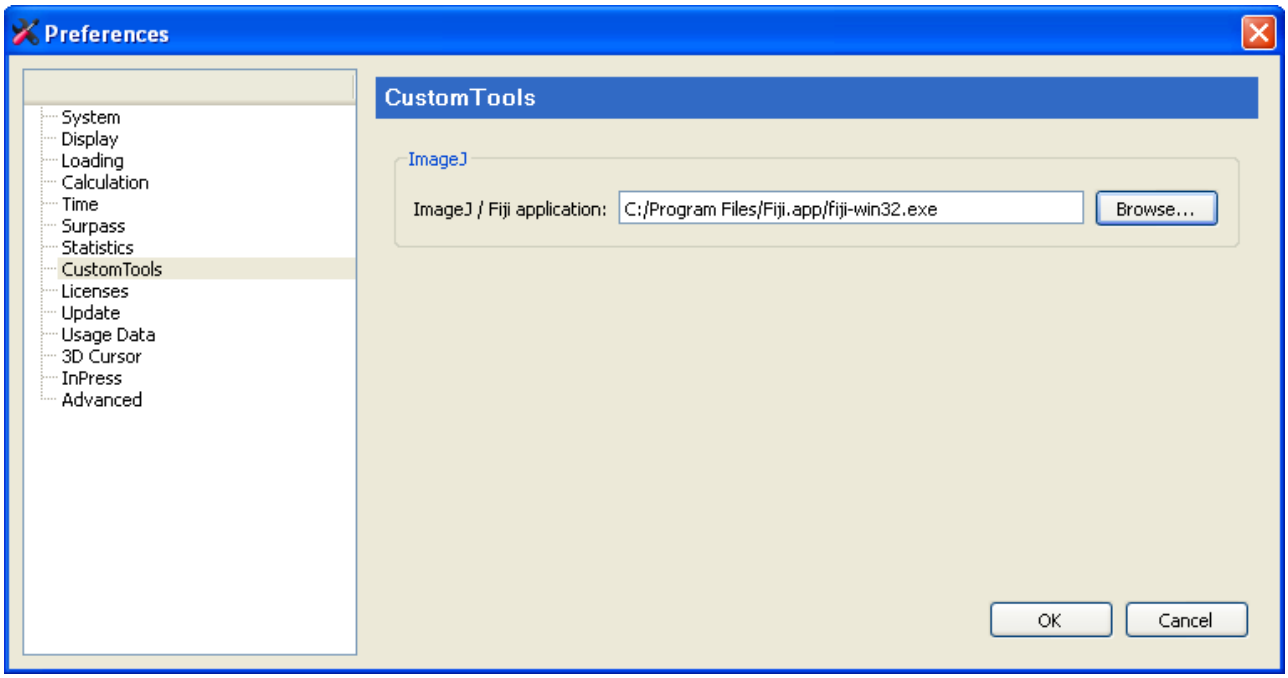
In this chapter you will learn how to integrate Fuji plugins in Imaris by means of the Fiji menu and how to establish connections between the Fiji and Imaris.

If you already have Fiji installed, skip down directly to the to the **Options…** section. If you don't have Fiji at all, you have to install it. The latest version can be downloaded from the following locations:
Fiji: http://pacific.mpi-cbg.de/wiki/index.php/Downloads

Depending upon your operating system select the appropriate link.
To complete download follow the specific download instructions.

Options…

The first step of integrating the Fiji plugins within Imaris is to specify the folder with the Fiji.exe file. To do that open the Fiji menu and choose the last item in the list **Options…**
The Custom Tools window opens. In the field Fiji application either type in the respective path or use the button Browse.
Specify the Fiji folder and select the .exe file. Click on the OK button.



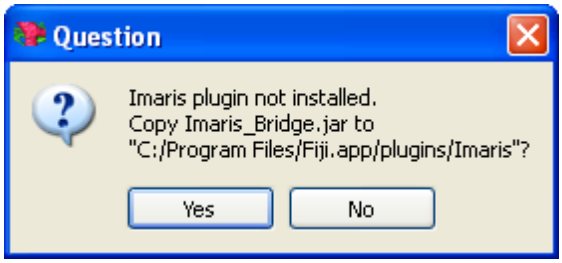
For Mac users:

/Applications/Fiji.app/Contents/MacOS/fiji-macosx

In the Fiji menu all options are now active.

6.2 Imaris Bridge.jar File

The downloaded plugins do not include the file needed to run your Fiji plugins in Imaris.
In order to run your Fiji plugins in Imaris you need to install the file, **Imaris_Bridge.jar file**; which provides the link between the two applications.
The installation starts automatically as soon as you select a plugin from the list.
The new window appears asking you to install Imaris_Bridge.jar file



You can confirm installation by selecting **Yes.**
The Imaris_Bridge.jar file is placed in a new subfolder, Imaris, within the Fiji application.

Security Alert window: Depending upon how your computer security is set, you may see a Security Warning. Select the option Unblock.

After installing the Imaris bridge file all plugins are ready to be used and no additional steps are required.

6.3 Configuration File

This section will show you how to expand the preexisting collection of plugins and embed initially unlisted plugins into the Imaris Fiji menu.

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You can customize the Fiji menu by the integration of new plugins.

Download plugin

To incorporate new Fiji plugin into Imaris, you are not required to modify, or access the Imaris code. Simply select a plugin from the Fiji list, download it and place it in the plugin menu or one of the submenus.

Configuration File

To properly run the plugin in Imaris it is important to create the **configuration file**.
The configuration file is needed to fully read/execute/and write permissions to the Fiji directory and subdirectories. The configuration file handles all the details of incorporating, executing and presenting the results of this new plugin within Imaris.

The configuration file has two main elements. The first is executed with Imaris. It has several lines that connect the plugin to Imaris. By adding a special header to the file you define the Menu or the Submenu and the name of the plugin in Imaris.
The second part is written as any plugin and executed by Fiji. This part of the file runs the Imaris-Bridge file, which provides a connection between the Fiji and Imaris, loads dataset information, performs some actions, sends images back to Imaris, closes Fiji and terminates the connection.
Once a file is created, copy the file to the Imaris Fiji plugins folder in the current Imaris Program Files path and you will have access to new plugins within Imaris.
The following is the example configuration file.

// <CustomTools>
// <Menu name="Fiji">
// <Submenu name="**submenu name** (e.g. Process)"> -This line is optional (embedding the plugin in the submenu)
// <Item name="**plugin name**" icon="Fiji">
// <Command>Fiji::**submenu name_plugin name** </Command> - Please make sure that the plugin name is the same as the configuration file name
// </Item>
// </Submenu>
// </Menu>
// </CustomTools>

call("Imaris_Bridge.In", getArgument());
run("**plugin name**");
call("Imaris_Bridge.Out", getArgument());
call("Imaris_Bridge.Terminate", getArgument());

Save the file, and copy it to the special folder:" **C:\Program Files\Bitplane\Imaris *version number* \imageJPlugins\configurations**

Please note: The Imaris version cannot be set differently than the currently running version.

Restart Imaris and the newly inserted plugin will appear in the Fiji menu.

Please note: Imaris Fiji compatible plugins are plugins that provide image data as a result.

6.4 Image From Fiji

Transfer the image to Imaris by selecting the Image From Fiji option.

6.5 Image To Fiji

Transfer the image to Fiji by selecting the Image to Fiji option.

6.6 Initially Installed Plugins

File
Open
Save

Image
Adjust
    Brightness/ contrast
    Color balance
    Window/Level

Process
Find Edges
Sharpen
If you would like to find out more about these plugins, additional information is available in the form the Fiji website.

Analyze
Color inspector 3D

Plugins
LOCI

Plugins are not only used to implement new processing functions, they are also used to support reading and writing file formats, which are not normally supported by Imaris.

Process

For the detail description it is recommend to visit the Fiji website.
There are a lot of examples and tutorials, which could help you to understand the features of these plugins.
http://pacific.mpi-cbg.de/wiki/index.php/Fiji's_Menu

Registration
    Correct 3D Drift
    Linear Stack Alignment with SIFT
Skeleton
    Analyze Skeleton
http://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:analyzeskeleton:start
    Skeletonize (2D/3D)
http://imagejdocu.tudor.lu/doku.php?id=plugin:morphology:skeletonize3d:start

6.7 Options...

Custom Tools
Here you can specify the Fiji folder. Either type in the respective path or use the button **Browse.**

6.8 Fiji Help

Select Help to directly open the Fiji chapter in the Reference Manual.

7 Menu Surpass

Please refer to chapter:

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Clipping Plane	Surpass View - Clipping Plane
External Object	Surpass View - External Object
Filament	Surpass View - Filament
Frame	Surpass View - Frame
Group	Surpass View - Group
Light Source	Surpass View - Light Source
Measurement Point	Surpass View - Measurement Point
Oblique Slicer	Surpass View - Oblique Slicer
Ortho Slicer	Surpass View - Ortho Slicer
Spots	Surpass View - Spots
Surfaces	Surpass View - Surfaces
Volume	Surpass View - Volume

Export Selected Objects...	
Delete Selected Objects...	Del

Switch View Mode	Esc

7.1 Export Selected Objects...

In the Surpass Tree highlight all Items to be exported. To export all Surfaces, Contours, and External objects, select the Export Selected Objects... .

- Select menu Surpass - Export Selected Objects... .

The Save selection as External Objects box is displayed.

- Select the directory and enter the name for the file to be saved and click **OK**. The object is saved as either as an Inventor file with the extension *.iv or as VRML2 file with extension *.vrml. The saved object can be loaded as External objects to another data set.

See also:
Surpass View - Overview - Scene File Concept
Surpass View - External Object

7.2 Delete Selected Objects ...

In the Surpass Tree highlight all Items to be deleted. To delete all Surfaces, Spots, Contours, and External objects, select the entire Surpass Scene folder.

- Select menu Surpass - Delete Selected Objects.

The Delete selection window with a confirmation question is displayed.

See also:
Surpass View - Overview - Surpass Tree - Objects toolbar - Button Delete ...
Surpass View - Overview - Scene File Concept

7.3 Switch View Mode

In the Surpass view there are two viewing modes: Select and Navigate.

Select

The cursor becomes an arrow. You use the pointer mode Select whenever you want to mark something in the image, e.g. to set some Measurement Points on the object surface.

Navigate
The cursor becomes two turning arrows. You use the pointer mode Navigate to move, rotate or zoom the image in the viewing area.

Tip: You can easily switch between the two pointer modes using the ESC-Key. The effect is directly visible on screen by the altered mouse pointer display.

See also:
Surpass View - Overview - Camera Toolbar (Pointer Select or Navigate)

8 Menu Help

Quick Start Tutorials	Ctrl + F1
Keyboard and Mouse	F1
Reference Manual	
Programming Interface	
License...	F2
Check for Updates now...	F3
Bitplane	
About Imaris...	

How to find the pdf based help for PC Users:

To open the pdf help file in Imaris first select **Start**- **All Programs** – **Imaris**.
The pdf version of the **Reference Manual** and **Quick Start Tutorials** are located there.

How to find the pdf based help for Mac Users:

To open the pdf help file in Imaris first locate and select the Imaris application file.
By default, the file is in the Application folder and the file name is Imaris 7.3.app.
With the right click select the option **Show Package Contents**.
The new window will open.
Select the **Contents** folder and with a double click select the **Shared Support** folder.
The pdf version of the **Reference Manual** and **Quick Start Tutorials** are located there.

See also:
Addendum - Mouse & Keyboard Mac

8.1 Quick Start Tutorials

The Quick Start Tutorials provide you with the basic information how-to-use Imaris but may also show yet unrecognized new features of the software to the advanced user.

The tutorials are designed to be followed sequentially, but if you are already familiar with Imaris the basic lessons may be skipped. The tutorials are cross-referenced by hyperlinks highlighted in blue underlined font. The Table of Contents and the Index provide further support for navigation in the tutorials.

Each tutorial is a hands-on seminar, therefore move Imaris to the left hand side of the screen and adjust the online tutorial on the right hand side of the screen and practice step-by-step.

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8.2 Keyboard and Mouse

Click on this menu entry and the Reference Manual opens in the actual browser. The page for the global Mouse & Keyboard functions displays.

Global Mouse & Keyboard Functions

For PC User:

Addendum - Mouse & Keyboard PC

For Mac User:

Addendum - Mouse & Keyboard Mac

8.3 Reference Manual

The Reference Manual provides you with the basic information about Imaris: The menu(s), the different Imaris views and all adjustable parameters in the program.

To find a specific parameter use the "Search function" in the online version of the Reference Manual.

Context Sensitive Help

For PC User:

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the massage the Reference Manual opens in the actual browser and displays the corresponding page.

For Mac User:

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the massage the Reference Manual opens in the actual browser and displays the corresponding page.

8.4 Video Tutorials

The entries in this menu automatically open eight Imaris video tutorials to help and improve your use of Imaris. To watch the video tutorial select the title from the list.

1. Introduction
2. Mixed Model Rendering
3. Simple Line Measurements
4. Automatic Dendrite and Spine Detection (FilamentTracer)
5. Semi Automatic and Manual Filament Tracing Methods
6. Edit Tracks
7. 3D Tracking
8. 2D Tracking

8.5 Programming Interface

Select Programming Interface to open the Imaris COM Interface Documentation for ImarisXT.

8.6 License...

Overview

Node-locked License
Node-locked licenses grant permission to run the licensed software on one computer (node) only. Node-locked licenses are the easiest way to license the software. You can use the License Administrator to directly download a node-locked license from the Bitplane server. If your computer is not connected to the Internet it is also possible to download this license from another computer and then copy it to the target machine.

Floating License
Floating licenses grant permission to run the licensed software on several computers. One floating license can activate only one instance of the program at any given time. Floating licenses require the installation of a separate license server that runs on a Windows computer. The server installation requires more technical know how.
The License Administrator application easily let you enable a floating license.

Demo License
A Demo License is a time-limited (usually one month) license. It is not an official licensing scheme and therefore can't be purchased. It's main purpose is for internal Testing and to grant customers with licensing issues an easy and quick workaround so that their productivity can continue until the problem is solved. Simply open the License Administrator application and open the Demo License you received from your local support representative.

Evaluation License
Evaluation Licenses are temporary licenses allowing customers to explore all fascinating possibilities Imaris offers before actually purchasing it. The license is only limited in time (20 days), but not in features. Additionally an Evaluation License is issued per user.
To get an Evaluation License you will have to register yourself first at:

http://www.bitplane.com/go/download/imaris-evaluation

An automatic mail response should provide you with your personal RegistrationID and its corresponding Passcode. These credentials can then be entered during the application's start up.

First-time Installation

If you have installed Imaris for the very first time, you have to set up a license before you can use Imaris.

A dialog window appears during the start up of the application.

The same dialog window appears if you have accidentally deleted the license .

You now have two options how to proceed:

• If you have a Node-locked or a Floating License you should use the License Administrator application for the further license setup. This is done by simply clicking on the “Start License Administrator” button. Please note that the License Administrator application needs administrative privileges.

• If you want to evaluate Imaris first, please register yourself first at:

http://www.bitplane.com/go/download/imaris-evaluation

The automatic mail response contains your RegistrationID and Passcode.
To enable an Evaluation License enter these values in the corresponding fields and click on the “Use Evaluation

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License” button.
Please note that a working internet connection is needed to start up Imaris in Evaluation mode.

Installation of additional Licenses

If you already have set up a license but you want to add other licenses as well, you can do this in the License Preferences dialog.

Once more, you'll have the option either to start the License Administrator application or to use an Evaluation License in addition if you would like to try out a new feature before buying the license.

License Administrator

Here you can adjust the default license settings.
License Administrator is an application designed to manage the licenses of various Bitplane applications. It provides a simple interface to add or delete any licenses. When a license is added, the license file gets copied to a system folder where it is secure from (accidental) manipulation by standard users. Although it is possible for administrators to modify license files directly in this folder we strongly advise you not to do so. It is recommended to use the License Administrator application instead.

License Administrator Button
This button starts the License Administrator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use the License Administrator to set up your licenses. Please note that you'll need administrative privileges in order to start the License Administrator.
Any change of licenses will require a new start of Imaris.

Except the Evaluation License which is bound to a single user, all other licenses are bound to the whole system. Therefore any license modifications need administrative privileges.

If you have access to an administrative system account, the License Administrator should just start normally.

Start License Administrator Button
This button starts the License Administrator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use the License Administrator to set up your licenses. Please note that you'll need administrative privileges in order to start the License Administrator.
Any change of licenses will require a new start of Imaris.

Evaluation License

Use Evaluation License Button
After you entered your RegistrationID and Passcode you can click on this button to enable the Evaluation License. This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the light bulb will turn on.
You can also disable your Evaluation License by clicking again on this button and the light bulb will turn off. This indicates you aren't using any Evaluation Licenses.
Any change of licenses will require a new start of Imaris.

RegistrationID
Please enter your RegistrationID you received by mail first.

Passcode
Please enter your Passcode you received by mail first.

Available Licenses

The table displays all existing Imaris licenses on the system and additional information for each feature:

Version
The latest Imaris version this license can be used with.
Type
Which type of licensing scheme is used. An overview is given at the very beginning of this page.
Status
Displays the status of a license.
Expiration
Shows the number of days until this license expires.
Expiration
Shows the number of days until this license expires.

License colors
In order to gain a quick overview, the validity of a license is also indicated by different colors:
– Green: License is valid.
– Yellow: License is valid, but all licenses are currently in use.
– Red: License has expired.
– No color: License is not available (e.g. this additional license feature hasn't been purchased).

You can select with which license(s) your Imaris should start per default. Any change will require a new start of Imaris.

Check/Uncheck All

Check this box to enable or disable all available Imaris licenses on the system.

Please note that the Feature “ImarisBase” can't be disabled because this would render Imaris unusable.

The default License Administrator executable can be found here:

Windows XP, Windows Vista and Windows 7:
32-bit: C:\Program Files (x86)\Bitplane\Imaris 7.3.0\LicenseAdministrator.exe
64-bit: C:\Program Files\Bitplane\Imaris 7.3.0\LicenseAdministrator.exe

Mac OS X:
/Applications/Imaris 7.3.0 beta3.app/Contents/MacOS/LicenseAdministrator

If you did not have set up a license yet (e.g. first-time usage) this window is empty. In that case, please use the **Add License Location** button to add a new license location.

Add License Location Button
Use this button to add new licenses. A description is given in the Add license Window chapter.

Delete License Location
If you click this button, the selected license location entry in the table underneath will be deleted. Before using this button, please make sure you selected the right table entry. In case of a node-locked license you are asked if you want to keep the license. It is recommended that you keep all license files.

License Location Table
This table lists all available license locations. Normally it would be just enough to have one license location defined. But there exist also good reasons to use multiple locations.

For example you might have a floating license server with just some basic features enabled. Maybe later you purchased some additional features in a node-locked licensing scheme. You can now just define both locations and the licensed features are merged on your system automatically.

The table shows also some additional Information:

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- Type: This is either a License Server (floating license scheme) or a License File (all other schemes)
- Number of valid / total Licenses: These numbers shows you how many different features your license contains and it lets you easily verify that all licenses are still valid.

If you click on another table entry, the License table underneath gets updated and shows you detailed information about all available features of the current selected license location.

Licenses Table
In this table you have a detailed overview of all licensed features of a license location. You can switch among different license locations by selecting the corresponding entry in the License Location table.

Each feature provides some information :
Version, Type, Status, Expiration
The validity of a license is also indicated by different colors.

Finish Button
This button is only enabled if you added at least one license location. It is an indicator when all necessary steps to set up a license are done. If all features in the above License table are highlighted in green (or only some of them) you successfully managed to add a license. If you click the button, it just closes the License Administrator and you can proceed using Imaris.

Add License Window

First, you have to select your licensing scheme:

Download Node-locked License:
If you decide to add a Node-locked License, it is checked if your computer is connected to the internet first. If the connection could be established successfully, the Online-Licensing Wizard is shown; if not, you have the possibility to download the license from another computer which is described in the Offline-Licensing Wizard.

Online-Licensing Wizard:
This wizard has three steps:

1. Select the Network Interface
You need to select the network interface which is bound to the license. If your Host-ID is already known to our server, the available product for installation will be shown in the table below.

2. Check your Order-ID for newly ordered Products:
If you have ordered new products, you have to enter the Order-ID in this step. Clicking on the “Check Order-ID” button will add the products to the table below. Once you checked an Order-ID, it is bound to the currently selected network interface.
If your Host-ID is already known to the system, the available products will be shown in the tables below and you could skip that step.

3. Select the Licenses for Installation
In this step you just have to mark all products you want to install by checking them in the left table.
The right table is for your information only and shows all currently installed products.
When you klick on the “OK” button, the license file is downloaded and the corresponding entry is added to the License Location table.

Offline-Licensing Wizard:
If you are not connected to the Internet, this could have two reasons: it could be that:
1. your computer is behind a web proxy server which omits a direct connection or
2. you could be intentionally unplugged .

Therefore you have the following choices:

A) Set up a Proxy Server
Enter your proxy server's name and its port and click on the **Test Connection** button. If the connection could be established you are automatically redirected to the Online-Licensing Wizard described above.
If not, either the credentials you entered are not correct or the connection is cut at another place in the network. In that case please contact your network administrator or Bitplane Support teams eusupport@bitplane.com or ussupport@bitplane.com.

If you want to delete a previously set up proxy server, just delete both fields and click on the button again.
The proxy server settings can also be altered in Imaris' system preferences dialog.

B) Download the Node-locked License from another Computer
You can download the license from another computer connected to the internet.
On that machine log on to http://www.bitplane.com/activation/acti_offline_ident.html
and enter the Host-ID you wish to bind to the license together with your Order-ID into the web form. The download of the license should immediately start and you could transfer the file afterwards to the offline computer (e.g. by using a USB stick).
This instructions can be saved in a file by clicking the **Save Instructions...** button.

When you have downloaded the license you can just open it with the **Open License...** button or enter its path directly into the field on the left of the button.
When you click **OK** the license file is copied to the proper location.

Define Floating License Server:
In order to add a Floating License Server, just enter the server's name and its port to the corresponding fields and click on the **OK** button. The default port is 2700.

Install License from Existing File:
If you received a Demo License from your local support representative or you need to add a license file directly you can use this wizard. If you click on the **Open License...** button you can browse to the license you want to add. You can also specify the license file to load by entering its path directly into the field left from the button. If you click on the **OK** button, the license is copied to the right location and it's added to your License Location table.

See also:
Menu Edit - Preferences... - Calculation
Menu Edit - Preferences... - Licenses
Menu Edit - Preferences... - Display

8.7 Check for Updates now ...

If your computer is connected to the Internet, Imaris can directly check, if a software Update is available.

8.8 Bitplane AG

If your computer is connected to the Internet, Imaris can directly open the Bitplane AG homepage (www.bitplane.com).

8.9 About Imaris...

The Imaris start screen displays.

Imaris
Version number and release date.

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European Patent Registry No. 05009677.7
Patent Pending.

Using XML parser expat: Copyright
1998, 1999, 2000. Thai Open Source
Software Center Ltd and Clark Cooper

www.bitplane.com
welcome@bitplane.com

9 Toolbars

In the standard Imaris view there are three different toolbars available. The Main Toolbar, the Status Bar and the Time Bar.

See also:
Surpass View - Overview - Camera Toolbar

9.1 Main Toolbar

	Please refer to chapter:
Open	Menu File - Open
Save	Menu File- Save
Save as	Menu File - Save as
Snapshot	Menu File - Snapshot
InPress	Menu File - InPress

The display modes Slice, Section, Gallery and Easy 3D are in one icon group. Click on the arrow to select the respective mode.

	Please refer to chapter:
Slice	Slice View
Section	Section View
Gallery	Gallery View
Easy 3D	Easy 3D View
Surpass	Surpass View
Animation	Animation
InMotion	InMotion
Batch	starts Imaris Batch Coordinator
Coloc	Coloc
Bitplane AG	Menu Help - Bitplane AG

9.2 Status Bar

At the bottom of the screen you find the Status Bar.

Zoom %
Imaris lets you enlarge or reduce the view of your data set with the Zoom. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, type a zoom value.

Mouse Clicks to Zoom

With a three-button mouse:

- Click with the middle mouse button on the image, hold down the middle mouse button, and drag the mouse up or down.
- Hold the Shift-key and click with the right mouse button on the image, and drag the mouse up or down.

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Button **100%**
Click on this button to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.

Button **Fit**
Click on this button to pan the position to best fit in the window and adjust the zoom factor.

Button **Full Screen**
Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window, click on the button Full Screen in the lower left corner.

Button **Navi**
Click on this button to toggle the display of the Navigation window of the data set in the upper right corner of the viewing area. If you move the data set, the overview updates in real-time. In the pointer mode Select you can click & drag the Navigation window to another position in the viewing area. The Navigation window is placed to the nearest corner.

Change Position of the Navigation Window

- In the pointer mode Select you can click & drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section view the position of the Navigation window is fixed (lower right corner) and can not be moved.

See also:
Menu View - Fit
Menu View - 100%
Menu View - Full Screen
Menu View - Navi
Section View
Surpass View - Overview - Camera Toolbar (Pointer Navigate/Select)

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9.3 Time Bar

If a time series is loaded, a Time Bar displays at the bottom of the main window. It contains a slider showing all Time Points in the series, a **Time Settings** button, **Play** and **Pause** button (arrow to the right), and a **Record** button (red dot).

The Time Bar is visible in the display modes Slice, Section, Gallery 3D Preview, and Surpass. It allows you to view a particular Time Point or to automatically play 4D images. The views change when selecting a new Time Point.

Play and Record Time Series

Button **Time Settings...**
Click on this button and you open directly the Time default parameter settings.

Button >
Click on this button to play the time series. To pause the continuous display through all the Time Points in the time series re-click on the button.

Button O
To record the time series as movie click on the button **Record** (red dot). The Save As Movie window is displayed (see below).

Slider
To inspect any the Time Point in the time series manually navigate the slider as follows (be sure that the Pointer is in the Select mode):

- Drag the slider handle to the left or right.
- Moving the slider to the left reduces the time point, moving it to the right increase time point.
- Select the slider handle with a left mouse click and then use the Arrow-keys to the left/to the right on the keyboard.
- Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first respectively last Time Point.

The respective Time Point number is displayed on the right hand side of the slider in the number field.

Window: Save as Movie

File name
Enter a file name.

Save as type
Select the desired file type (QuickTimeMovie, TIFF series, or AVI).

Movie Settings

Compression Factor
Select a compression factor between 0 (High Quality) and 100 (Low Quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. Moving the slider to the left reduces the compression point, moving it to the right increase compression pint. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate
Define how many frames are displayed per second.

Add to ImageAccess database
Check the box to add the file to the ImageAccess database.

Play Movie with default Media Player when finished
Check the box to play the movie with the default media player when finished.

QTVR Settings

The QuickTimeVR Settings are not available.

See also:
Menu Edit - Preferences ... - Time (default parameter settings)
Surpass View - Overview - Camera Toolbar (Pointer Select)
Surpass View - Overview - Camera Toolbar - QuickTimeVR

10 Slice View

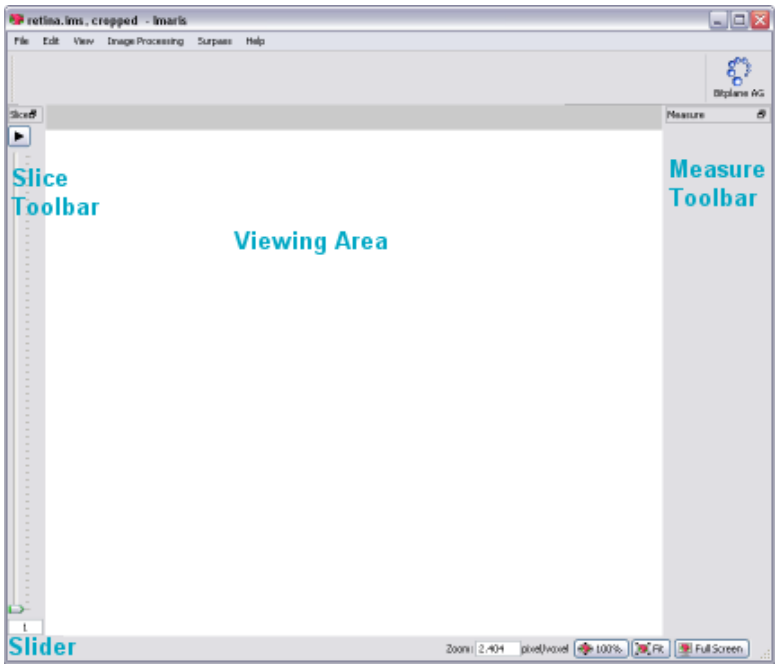
How to Open the Slice View?

- Click on the icon in the Main toolbar. If the icon Slice is not displayed click on the arrow in the group: Section, Gallery, Easy 3D, and select Slice.
- Select menu View - Slice.
- Press the key combination Ctrl + 1.

See also:
Slice View - Mouse & Keyboard Mac

10.1 Overview

The Slice view mode is the view in which an image is displayed when loading a data set into Imaris. The Slice representation shows a 2D view from the top in the z-axis. It shows all images in the data set along the z-axis, so you can inspect any slice along the z-axis for a general overview of the data set.



When moving the cursor over the image, the current voxel intensity and the 3D position in the data set (in

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brackets) is displayed in the lower left corner of the view.

Additional Information in the Status Bar

The Slice view also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

Ch 1 ... n at (X, Y, Z)
The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:
Toolbars - Status Bar

10.2 Slice Toolbar

Slider

To inspect any slice in the data set along the z-axis you use the Slider. Navigate the slider as follows:

- Drag the slider handle in the left control bar up and down.
- Select the slider handle with a left mouse click and then use the Arrow-keys up/down on the keyboard.
- Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first respectively last slice.
- Select the slider handle with a left mouse click and then press the PageDown-key or the PageUp-key to display the first respectively last slice.
- Enter the desired slice number in the number field at the bottom of the slider.

Button >
Click on the **Play/Pause** button to see a continuous display through all the slices in the stack. To pause re-click on the button.

Slice Number Field
The respective slice number is displayed on the bottom of the slider in the number field.

10.3 Measure Toolbar

Type

Line
Set the two Measurement Points with two consecutive clicks. The result is displayed in the status field Distance.

Polygon
All Measurement Points are consecutively connected by lines and the displayed distance is the sum of the distances between the points.

Grid
Check this box to display a grid in the image.

Distance
The result of the measurement is displayed in the status field distance.

Button **Delete**

Click on **Delete** to clear the status field and start a new measurement.

10.4 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Slice View

Ctrl + 1	Activates the Slice view
Left-click	Set a measurement point
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard PC

10.5 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Slice View

Command + 1	Activates the Slice view
Click	Set a measurement point

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard Mac

11 Section View

How to Open the Section View?

- Click on the icon in the Main toolbar. If the icon Section is not displayed click on the arrow in the group: Slice, Gallery, Easy 3D, and select Section.

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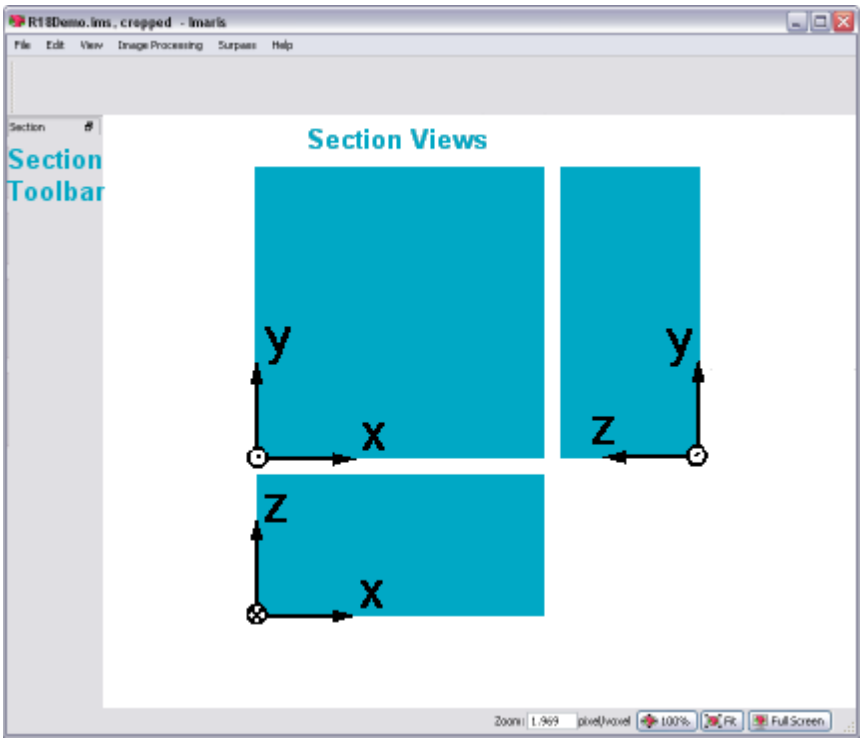
- Select menu View - Section.
- Press the key combination Ctrl + 2.

See also:
Section View - Mouse & Keyboard Mac

11.1 Overview

The Section view lets you inspect the environment of any given point within the data set by showing the cut through the point in the x-, y-, and z-axes or a defined portion of the data set (several slices together).

The illustration below shows the coordinates in the three different display areas of the section view.



To select a point, click in any of the three views, hold the button down & drag the crosshairs to the requested point. The crosshairs immediately snap to the cursor when clicking anywhere in the image. The other views, connected by one coordinate, automatically follow the movements.

Additional Information in the Status Bar

The Section view also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

Ch 1 ... n at (X, Y, Z)
The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:
Toolbars - Status Bar

11.2 Section Toolbar

View

Normal
Selects a single cut through the data set.

Extended
Additional colored lines are displayed in the viewing area. If Extended is checked the parameter sets Crosshair, Size and Mode are available.

Crosshair

The parameter set Crosshair is only available, if Extended is checked in the parameter set View (see above).

Normal
You may eliminate the Crosshair display on the image by unchecking the box. It is then only visible at the edges.

Extended
Select a portion of the data set in the x-, y-, and z-direction to be displayed. In the main screen, separate the two crosshairs. The planes between the crosshairs are rendered in the views showing the other axis. This allows you to see only certain interior structures without both the top and the bottom.

- The image shows the white crosshair line together with colored focus range lines.
- The focus range can be set in any of the three directions as follows: Move the cursor on a colored line. The cursor changes its form to a straight arrow with a bar.
- Drag the line in the respective direction. The range is extended or reduced. Its depth is reflected in the x-, y-, and z-fields in the Size display. The Size values are indicated in the data set's dimensions, e.g., in µm. It can also be adjusted directly by entering values in the Size fields (see below) or by means of their up and down arrows.

Uncheck the Crosshair – Normal and/or Crosshair – Extended boxes to eliminate the crosshair and/or the focus range lines display on the image.

Size fields

The parameter set Size is only available, if Extended is checked in the parameter set View (see above). Display of the actual x-, y-, and z-position of the extended Crosshair. The positions can also be adjusted directly by entering values in the fields or by means of their up and down arrows.

Mode

The parameter set Mode is only available, if Extended is checked in the parameter set View (see above). Here you may choose the best display mode for the slice portion.

MIP
Calculated maximum over all slices in the range.

Mean
Calculated mean value over all slices in the range.

Blend
Calculated upon the transparency of the slices.

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11.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Section View

Ctrl + 2	Activates the Section view
Left-click	Set the section center
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard PC

11.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Section View

Command + 2	Activates the Section view
Click	Set the section center

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard Mac

12 Gallery View

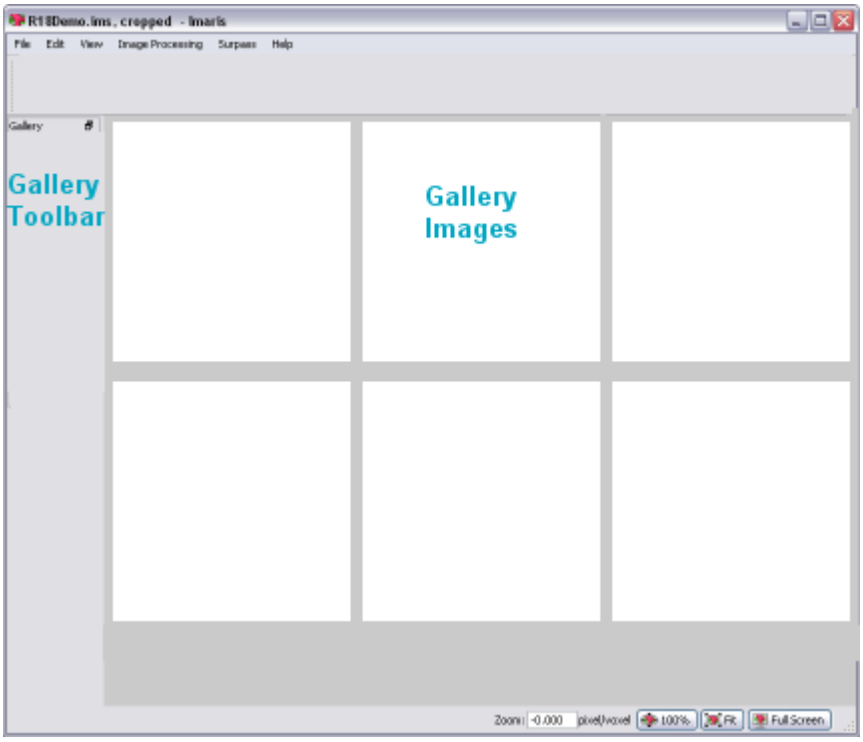
How to Open the Gallery View?

- Click on the icon in the Main toolbar. If the icon Gallery is not displayed click on the arrow in the group: Section, Slice, Easy 3D, and select Gallery.
- Select menu View - Gallery.
- Press the key combination Ctrl + 3.

See also:
Gallery View - Mouse & Keyboard Mac

12.1 Overview

The Gallery view displays all available slice images of the data set. In the Gallery view you can adapt the display to your needs to gain more information from the slice series. When the Gallery view is selected, all slices are displayed. Click on images to select or deselect them. Selected images are marked with a colored frame. Holding the Shift-key lets you select a continuous series of images. Hold the Ctrl-key to select various images spread over the gallery.



Additional Information in the Status Bar

The status bar indicates which of how many available pages is currently being displayed, and how many slices have been selected. Changes in the Gallery view only affect the current display and do not crop the data set.

See also:
Toolbars - Status Bar

12.2 Gallery Toolbar

In the Gallery view the following display and selection controls are available:

Slice

By clicking on the arrows, you move up and down the images in the gallery one by one. This is especially useful whenever the display on the screen does not include all the slices.

Page

If there are more images than displayed on screen, move up and down the pages by clicking on the arrows or

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go directly to the first or last page by using the respective buttons.

Columns

Specify the number of columns to be currently displayed on the screen by clicking on the up/down arrows or by entering the requested number.

Skip

Select every second image from the data set by selecting 1 image to skip, or every third image by selecting 2 images to skip (or any other skip interval analogously, by clicking on the up/down arrows or entering the appropriate number directly).

Display

Button **Select...**
Highlight image(s) and click on the button **Select...** . Only the selected image(s) is(are) displayed in the gallery. Re-click on the button **Select...** and all images are displayed in the Gallery.

Slice Number Display

The slice number is displayed beneath each slice.

12.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Gallery View

Ctrl + 3	Activates the Gallery view
Left-click	Select slice
Ctrl + left-click	Add single slice to selection
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard PC

12.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Gallery View

Command + 3	Activates the Gallery view
Click	Select slice
Shift + click	Add slices to selection
Command-click	Add single slice to selection

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences… .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard Mac

13 Easy 3D View

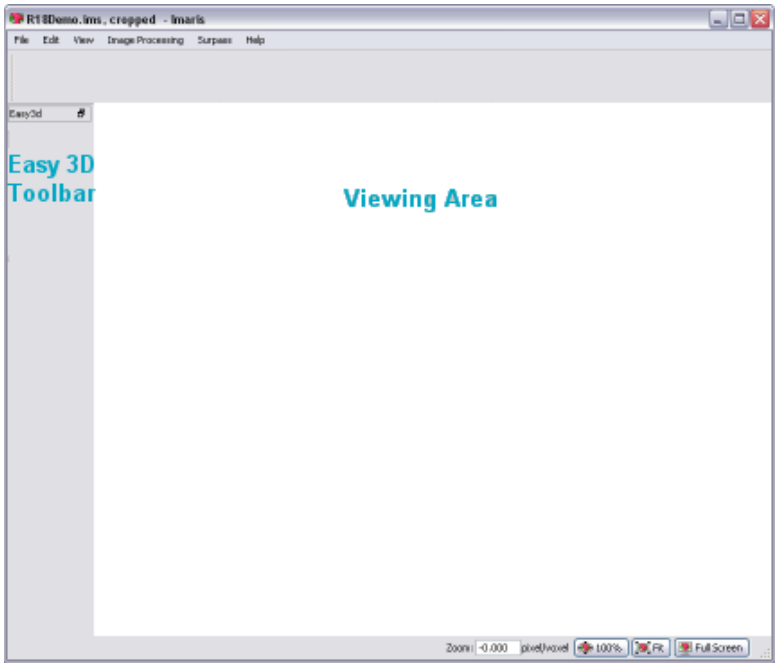
How to Open the Easy 3D View?

- Click on the icon in the Main toolbar. If the icon Easy 3D is not displayed click on the arrow in the group: Section, Gallery, Slice, and select Easy 3D.
- Select menu View - Easy 3D.
- Press the key combination Ctrl + 4.

See also:
Easy 3D View - Mouse & Keyboard Mac

13.1 Overview

The Easy 3D view provides a quick fixed view with a few parameters for rendering and light setting. This lets you explore the data set in the 3D mode before doing any further processing.



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13.2 Easy 3D Toolbar

Rendering

MIP
(Maximum Intensity Projection) - Shows the maximum intensity of all layers along the viewing direction.

Blend
Shows the color obtained by blending all values along the viewing direction and including their transparency. If Blend is selected, the parameter set Light is available (see below).

Light

If Blend is selected (see above), the parameter set Light is available.

Light
Check the box to switch the light on and adjust the following parameters. There are two predefined light setting parameters in Easy 3D Blend to choose from.

Left
Light from the left, shadow to the right.

Right
Light from the right, shadow to the left.

Button **Settings...**
In Blend mode, with light enabled, click the button **Settings...**. The Easy 3D Settings dialog box displays (see below).

Calculate

Auto
The image is calculated and displayed automatically.

Manual
The image calculation starts after you click on the button **Calculate**. If you select Manual as calculation mode, the button **Calculate** is available.

Button **Calculate**
Click on this button to start the calculation for the Easy 3D image view. The button is only available, if you select Manual as calculation mode.

Window: Easy 3D Settings

Light

Directional Intensity
Controls the brightness of structures that are illuminated from the light source.

Ambient Intensity
Controls the brightness of structures that are in the shadow. The default (0) means that structures completely in the shadow are black. The higher the value the brighter the picture and the lower the shadowing contrasts.

Channel Opacity Combination

Allows you to control the opacity of the image.

Opaque
Translucent
Custom
Select Opaque, Transparent, or Custom opacity. It affects only the behavior of interleaving structures of two or more channels.

13.3 Mouse & Keyboard PC

Mouse & Keyboard Functions in the Easy 3D View

Ctrl + 4	Activates the Easy 3D view
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard PC

13.4 Mouse & Keyboard Mac

Mouse & Keyboard Functions in the Easy 3D View

Command + 4	Activates the Easy 3D view

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:
Addendum - Mouse & Keyboard Mac

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14 Surpass View

The Surpass view provides extended functions for visualization, including:

- A large viewing area with numerous tools for data preparation, presentation and manipulation.
- A selection of different types of data display as well as any combination of them.
- The possibility of loading additional External objects for comparison.
- Grouping functions.

How to Open the Surpass View?

- Click on the icon in the Main toolbar.
- Select menu View - Surpass.
- Press the key combination Ctrl + 5.

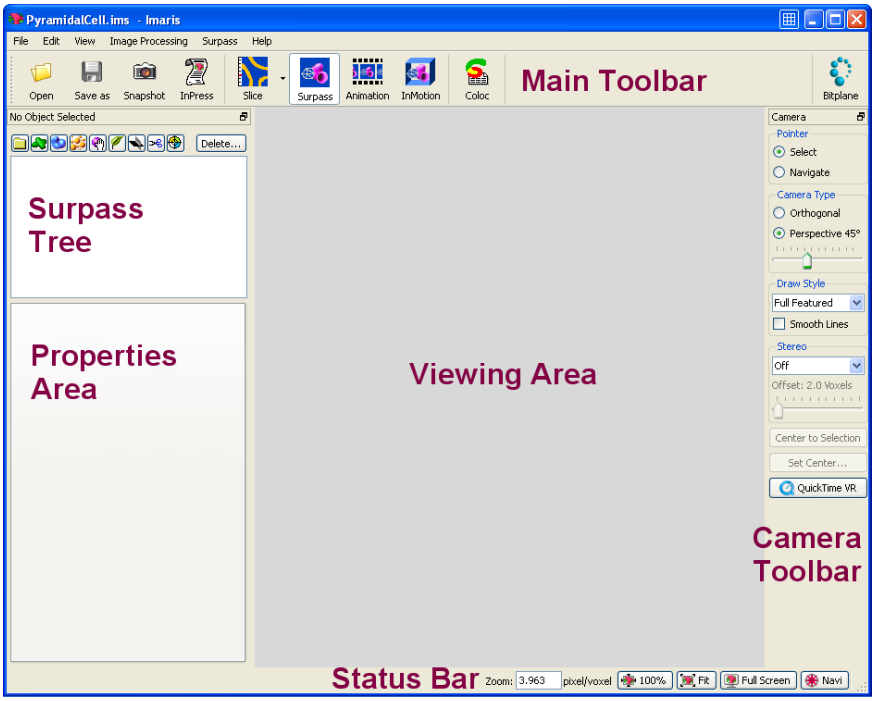
Save and Load Surpass Configuration

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Surpass Scene and can be stored in a Scene file with the extension *.imx. The Surpass Scene can be loaded again to the same data set or to another data set. For details please refer to chapter Surpass View - Overview - Scene File Concept.

See also:
Surpass View - Overview - Mouse & Keyboard Mac
Surpass View - Ortho Slice
Surpass View - Cells
Surpass View - Clipping Plane
Surpass View - Filament
Surpass View - Frame
Surpass View - Group
Surpass View - Measurement Point
Surpass View - Oblique Slicer
Surpass View - Ortho Slicer
Surpass View - Light Source
Surpass View - Volume
Surpass View - Surfaces
Surpass View - Spots

14.1 Overview

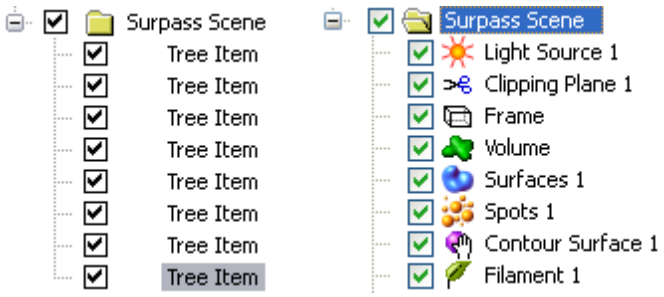
The Surpass main screen consists of six different areas:



- On top you find the Main Toolbar.
- On the left hand side you find the Surpass Tree and the Properties Area. In the Properties Area you find all available parameters for the selected Surpass Tree Item. The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.
- The Viewing Area displays all objects that are added to the Surpass Tree and are checked visible.
- The Camera Toolbar includes a number of tools for data preparation, presentation and manipulation, most of which are displayed as buttons.
- On the bottom you find the Status Bar.

14.1.1 Surpass Tree

The Surpass Tree displays a tree list of all Surpass Tree Items that were added to the viewing area.



Structure

The tree list is automatically generated and updated when a Surpass Tree Item is added or deleted. The first added object generates a group (Surpass Scene). All following new objects are stored in this group. A name is generated automatically for each Surpass Tree Item. To change the name, double-click on the item and enter a new name. Move objects or groups from one group to another by dragging and dropping them with the left mouse button.

How to Add a new Surpass Tree Item?

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All available Surpass Tree Items are available in the menu Surpass. To add new Surpass tree item click on the icon.

You find a list of all Items in the chapter Surpass View - Overview - Properties Area.

Display

Each Surpass Tree Item includes a check-box. Check the box to make the object visible in the viewing area. Uncheck the box to make the object invisible in the viewing area. The currently active object in the viewing area is highlighted in the Surpass Tree.

Groups

You can group objects into so-called component groups. Functions applied to the component group apply to all of its members. This facilitates the application of colors or the deletion of objects.

Please note: If Group folder is checked invisible, all Items in the folder are invisible.

Multiple Selection

You may select more than one listed Item at a time for an operation. The selection functions in Surpass correspond to the Windows functions:

- Consecutive: Press and hold the Shift-key down and select the first, then the last entry to be selected from the list. All entries in between the two are also selected.
- Selective: Press and hold the Ctrl-key down and select any required entries from the list.

All selected entries are highlighted and commands or operations apply to all of them.

Objects Toolbar

In the Objects toolbar you find a selection of Surpass Tree Items. To customize the Objects toolbar please refer to chapter Menu Edit - Preferences... - Surpass (Object Creation Buttons).

Button **Delete...**
To delete Surpass Tree Item, highlight the Item in the Surpass Tree and click the button **Delete...** . The Delete selection window with a confirmation question is displayed.

Naming Conventions

Objects are automatically named by Surpass as follows:

Cells	Cells n
Clipping Plane	Clipping Plane n
Contour Surface	Surface
External Object	External Object n
Filament	Filament n
Frame	Frame
Group	Group n
Surfaces	Surface n
Light Source	Light Source n
Measurement Point	Measurement Points n
Oblique Slicer	Oblique Slicer

Ortho Slicer	Ortho Slicer n
Spots	Spots n
Volume	Volume (only one volume can be created)

Surpass Tree Item Properties

Each Surpass Tree Item has its own set of adjustable parameters. They are displayed in the properties area.

Surpass Tree Item Tabs

Each Surpass Tree Item has its own set of adjustable parameters. They are grouped in different Tabs.

Save and Load Surpass Tree Configuration

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Surpass Scene and can be stored in a Scene file with the extension *.imx. The Surpass Scene can be loaded again to the same data set or to another data set. For details please refer to chapter Surpass View - Overview - Scene File Concept.

See also:
Menu Edit - Preferences... - Surpass (Object Creation Buttons)
Menu Surpass - Delete Selected Objects...
Surpass View - Overview - Properties Area
Surpass View - Group

14.1.2 Scene File Concept

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Surpass Scene and can be stored in a Scene file with the extension *.imx. The Surpass Scene can be loaded again to the same data set or to another data set.

Export Scene File

The Scene File can be written as follows:

- Select menu File - Export Scene in the Imaris menu bar.

The Save Imaris Scene window is displayed. Select the directory and enter the Scene File name and click **OK**. The Surpass Tree Items are saved as Imaris Scene File with the extension *.imx.

Import Scene File

Any Scene File with the file extension ***.imx**, ***.imod** or ***.mod** can be loaded into the Surpass view for further viewing.

- Select menu File - Import Scene..... in the Imaris menu bar.

The Load Surpass Scene window is displayed. Select the directory and requested file to be loaded, and click **OK**. The Scene File is loaded.

See also:
Menu File - Import Scene...
Menu File -Export Scene...

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Surpass View - Overview - Surpass Tree
Surpass View - Group

14.1.3 Properties Area

The Properties Area displays all available parameters for the selected Surpass Tree Item.

Surpass Tree Item - Properties
The name of the heading is a combination of the selected Surpass Tree Item, followed by "- Properties". If you select another Surpass Tree Item the heading changes accordingly.

Tab X
The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.

List of available Tabs:

Surpass Tree Item	Parameter Tab
Cells	Tab Create see Surpass View - Cells Creation Wizard Tab Settings Tab Creation Tab Edit Tab Filter Tab Statistics Tab Color
Clipping Plane	Tab Settings
External Object	Tab Settings Tab Color
Filament	Tab Create see Surpass View - Filament Creation Wizard Tab Settings Tab Creation Tab Draw Tab Edit Tab Filter Tab Statistics Tab Color
Frame	Tab Settings Tab Color
Group	Tab Settings Tab Color Tab Statistics
Light Source	Tab Settings Tab Color
Measurement Point	Tab Settings Tab Edit Tab Intensity Tab Statistics Tab Color
Ortho Slicer	Tab Settings

Oblique Slicer	Tab Settings
Spots	Tab Create see Surpass View - Spots Creation Wizard Tab Settings Tab Creation Tab Edit Tab Filter Tab Statistics Tab Color
Surfaces	Tab Create see Surpass View - Surfaces - Creation Wizard Tab Settings Tab Creation Tab Draw Tab Edit Tab Filter Tab Statistics Tab Color
Volume	Tab Settings Tab Draw Tab Statistics

See also:
Surpass View - Overview - Surpass Tree

14.1.4 Creation Wizards

A Creation Wizard is a user interface element where you are led through a sequence of dialogs. In a Creation Wizard you are forced to perform a task in a specific sequence.

Examples for Creation Wizards in Imaris are the Cells Creation Wizard, the Filament Creation Wizard, the Spots Creation Wizard, and the Surfaces Creation Wizard. Once you insert an new Item Filament, Spots, Cell or Surfaces you are automatically on the Tab Create in the Properties Area.

See also:
Surpass View - Cells - Creation Wizard
Surpass View - Filament - Creation Wizard
Surpass View - Spots - Creation Wizard
Surpass View - Surfaces - Creation Wizard

14.1.5 Viewing Area

Pan

To move the image within the Surpass view (pan the object) choose the mouse pointer mode Navigate. Click and hold the right mouse button while dragging the mouse. Release right mouse button to place the image.

On a PC or with a three-button mouse or on a Mac:

Right-click & drag	Pan image

On a Mac with a one-button mouse:

Ctrl + click & drag	Pan image

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Rotate

Rotating an image allows to change the viewing angle on a three-dimensional object.

Choose the mouse pointer mode Navigate. Click with the left mouse button in the image and hold the button down while moving the mouse (hold left + drag). The image on screen is rotated towards the direction the mouse is dragged. Be sure to hold the left mouse button down during the whole rotation. Stop moving the mouse and release the left mouse button to stop the rotation.

On a PC or with a three-button mouse or on a Mac:

Left-click & drag	Rotate image

On a Mac with a one-button mouse:

Click & drag	Rotate image

How to Keep the Image Continuously Rotated?

Choose the mouse pointer mode Navigate. Click with the left mouse button in the image and hold the button down while you move the mouse (hold left + drag). The image on screen is rotated towards the direction the mouse is dragged. Release the left mouse button while still dragging the mouse. The result is a continued rotation (speed of the rotation according to prior mouse motion). To stop the continued rotation re-click in the image area.

Zoom

In the Surpass view you zoom the image either by using the mouse or by selecting one of the buttons in the Status Bar at the bottom of the screen.

Using the Mouse

Choose the mouse pointer mode Navigate. To zoom in on the image hold the middle mouse button and drag it towards you. To zoom out from the image hold the middle mouse button and drag it away from you.

On a PC or with a three-button mouse or on a Mac:

Middle-click & drag	Move up: zoom out Move down: zoom in

On a Mac with a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in

Using the Buttons in the Status Bar

Zoom %
Imaris lets you enlarge or reduce the view of your data set with the Zoom. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, just type a zoom value.

Button 100%
Click on this button to rotate the image to the original position, center the image in the middle and set the zoom factor to one pixel per voxel.

Button **Fit**
Click on this button to pan the position to best fit in the window and adjust the zoom factor.

Button **Full Screen**
Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window re-click on the button **Full Screen** in the lower right corner.

See also:
Menu View
Toolbars - Status Bar
Surpass View - Overview - Camera Toolbar (Pointer Navigate)

14.1.6 Camera Toolbar

Pointer

Select
The cursor becomes an arrow. You use the pointer mode Select whenever you want to mark something in the image, e.g. to set some Measurement Points on the object surface.

Navigate
The cursor becomes two turning arrows. You use the pointer mode Navigate to move, rotate or zoom the image in the viewing area.

Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.

Camera Type

Orthogonal
Orthogonal display using parallel lines.

Perspective X
Perspective projection is a type of drawing, or rendering, that graphically approximates on a planar (two-dimensional) surface. If you select Perspective the slider (see below) is active.

Slider X
If you select as camera type Perspective X (see above) the slider is active. Drag the slider to adjust the vertical aperture angle of the camera.

Draw Style

Select the draw style of the object from the drop-down list.

Full Feature
Shows all objects as they are.

Wireframe
Draws Surface objects, Surfaces, Spots, Filaments, and Measurement Points as red colored wireframe models.

Hidden Lines
Draws Surfaces as wireframe models and hides all background lines.

Please note: Set Volume and OrthoSlicer objects invisible before selecting Hidden Lines.

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No Texture
Draws objects without textures.

Bounding Box
Shows only the boxes surrounding the objects.

Wireframe Overlay
Lays a red wireframe model over Surface objects, Surfaces, Spots, Filaments, and Measurement Points.

Low Resolution
Not yet implemented.

Points
Draws Surfaces as a point model.

Smooth Lines
Check the box to freeze the object in the view area and to prevent movement.

Stereo

Off
No stereo display in the viewing area.

Red/Cyan Anaglyph
This display mode requires colored glasses.

Quad Buffer
This display mode requires shutter glasses.

Interleaved Rows
This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is molded to have the form of dozens of tiny lenses or prisms per inch.

Interleaved Columns
This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is molded to have the form of dozens of tiny lenses or prisms per inch.

Offset
Display of the offset (0…5).

Slider
Adjust the offset to get an optimized 3D effect. Use a small offset if you are far away from the screen, use a big offset, if you are close to the screen. Click on the slider handle and move it to the desired position.

Button **Center to Selection**
Click on the button **Center to Selection** to move the selection in the center of the viewing area.

Button **Set Center...**
Use this button to select a new center of rotation. Click on the button **Set Center...** and then onto the Surpass Scene to define the new center on which the camera zooms in.

Please note: Rotation centers can be set on Surfaces, Contour Surface, Ortho Slicer and External objects

Button **Quick Time VR**
Please refer to the next chapter: Surpass View - Overview - QuickTimeVR.

14.1.7 QuickTimeVR

You find the **Quick Time VR** button in the Camera toolbar on the right hand side of the Surpass View.

Button **Quick Time VR**
QuickTimeVR animations show a display rotating around the horizontal or vertical axis, allowing a choice of the direction and the angle of rotation. Click on this button to open the Save As Movie window.

Window: Save as Movie

File name
Enter a file name.

Save as type
The file type is QuickTimeVRMovie (*.mov).

Movie Settings

Compression Factor
Select a compression factor between 0 (High Quality) and 100 (Low Quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate
Define how many frames are displayed per second. The quality of the animation depends directly on the number of frames presented in the rotation. More frames result in a slower and more fluid movement of the object. Rendering is a time-consuming process and requires about 1 minute per frame, depending on your computer and the complexity of the image. We recommend checking how long it takes to render a few images before specifying a large number of frames.

Add to ImageAccess database
Check the box to add the file to the ImageAccess database.

Play Movie with default Media Player when finished
Check the box to play the movie with the default media player when finished.

QTVR Settings

Here you define the degrees between two frames.

Total Angle Horizontally
Defines the angle of the horizontal movement, the default setting is 360.

Total Angle Vertically
Defines the angle of the vertical movement, the default setting is 180.

See also:
Toolbars - Time Bar (Play and Record Time Series)
Surpass View - Overview
Surpass View - Overview - Camera Toolbar
Animation - Animation Window
Animation - Save Animation

14.1.8 Mouse & Keyboard PC

General Mouse & Keyboard Functions in the Surpass View

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Ctrl + 5	Activates the Surpass View
Esc	Switch mouse mode Navigate/Select

In the Surpass Tree

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

Mouse in Select Mode

Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Alt	Temporary activate Navigate mode (until key is released)
Left-click	Select object
Ctrl + left-click	Add object to selection/remove object from selection

Mouse in Navigate Mode

S	Set center (on Surfaces, Contour Surface, Ortho Slicer and External objects)
Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Left-click & drag	Rotate image (scene)
Right-click & drag	Pan image
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in

See also:
Addendum - Mouse & Keyboard PC

14.1.9 Mouse & Keyboard Mac

General Mouse & Keyboard Functions in the Surpass View

Command + 5	Activates the Surpass View
Esc	Switch mouse mode Navigate/Select

In the Surpass Tree

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

Mouse in Select Mode

Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Click	Select object
Command-click	Add object to selection/remove object from selection

Mouse in Navigate Mode

S	Set center (on Surfaces, Contour Surface, Ortho Slices and External objects)
Arrow left	Go to the next time point
Arrow right	Go to the previous time point

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image
Click & drag	Rotate image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences… .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image
Click & drag	Rotate image

See also:
Addendum - Mouse & Keyboard Mac

14.2 Cells

ImarisCell is an Imaris module designed specifically to identify, segment, track, measure and analyze Cell, Nucleus and Vesicles in the 2D and 3D time-resolved images. ImarisCell provides simultaneous detection, measurement and analysis of cell objects combined with the interactive identification in the viewing area.
ImarisCell calculates and displays detailed statistics values for all the cell's components and their tracks. Interactive image exploration, simultaneous measurement and analysis of the many features in the image provide quick, accurate, quantitative and reproducible results.

How to Add new Cells?

- Select menu Surpass - Cells.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Cells - Properties.

See also:
Surpass View - Cells - Creation Wizard

14.2.1 Creation Wizard

In the first window of the Cell Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.
The most convenient way to apply the same creation parameters to different data sets is to use the **Load Parameters** option.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the manual Cells creation.

Depending on the chosen algorithm setting the Creation Wizard consists of the following steps:

Cell Body **Cell Membrane**

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Algorithm	Algorithm
Region of Interest (ROI)	Region of Interest (ROI)
Detection	Detection
Threshold	Threshold
Detect Nuclei Seed Points	Detect Nuclei Seed Points
Classify Nuclei Seed Points	Classify Nuclei Seed Points
Classify Cell	Classify Nuclei
Detect Vesicles	Membrane Detection
Classify Vesicles	Classify Cell
Tracking	Detect Vesicles
Classify Tracks	Classify Vesicles
Complete ROI	Tracking
	Classify Tracks
	Complete ROI

For further details please refer to Chapters Algorithm, Region of Interest, Detection, Threshold, Detect Nuclei Seed Points, Classify Nuclei Seed Points, Classify Cell, Classify Nuclei, Membrane Detection, Detect Vesicles, Classify Vesicles, Tracking, Classify Tracks, and Complete ROI.

14.2.1.1 Algorithm

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the Edit options.

Load Parameters

This option lets you use the identical creation parameters for multiple datasets.
The option to Load Parameters is only available if the set of the creation parameters has already been saved under the Creation tab using the Remember Parameters button.
Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Load Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

If you do not want to use the automatic Cell creation wizard select the **Skip automatic creation, edit manually** button. If you do not want to use the automatic Cells creation wizard select the **Skip automatic creation, edit manually** button.

Algorithm Settings

Depending on the used cell staining techniques and sample preparation, you can select either the **Cell Body** or **Cell Membrane** algorithm setting.

Select the segmentation algorithm which is applicable:

- for Cell-body stained images select the Cell Body algorithm

- for Cell Membrane stained images select the Cell Membrane algorithm

Segment only a Region of Interest

A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.
Check this box if you want to segment only a Region of Interest. If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally
This option appears only, if Segment only a Region of Interest is checked.
Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters (Complete ROI). Un-check this box and only the region of interest is processed.

Track Cells (over Time)
Check this box if you want to track the resulting Cells over time, for details please refer to the Tracking Tab.

Cells Wizard Buttons

Button Next
Press **Next** to proceed with the next window of the Creation Wizard.

Button Finish
Click on **Finish** to calculate the desired Cells and display the Cells objects in the viewing area.

Button Cancel
If you do not want to create the Cell objects click on **Cancel**.

14.2.1.2 Region of Interest

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

X, Y, Z, Time
Min, Max, Size
Minimum, maximum and **Size** of ROI coordinate values can be specified either by entering the value in the value fields or by changing the values by clicking on the up-and down arrows on the right hand side of the value fields.
Alternatively the location and size of the ROI can be adjusted directly in the viewing area. Size of the ROI:
1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X ,Y and Z.
Location of the ROI:
1.Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.
The numbers in the value fields are updated automatically.

Memory Usage:
Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button **Add**
Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button **Delete**
Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

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Total Memory Usage:
Display of the memory usage for the image size within the ROI.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.3 Detection

Cell Detection

Source Channel

Select the respective source channel, from which the Cells are to be computed.

Smooth

Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.
Smoothing may require a great deal of memory if the dataset is very large. Uncheck the check box or use smaller ROI if your system does not have enough memory.
If the option Smooth is selected then the Filter Width value should be determined. Use either the automatically given value or type in a new value in the data field.

Filter Width

The value of the Filter Width determines the Gaussian filter width.
The value is either automatically calculated from the voxel sizes (oriented at the largest voxel dimension) or it can be directly entered into the numerical field. The value calculates image coordinates and not voxels fraction .

Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.
The width of the Gaussian filter is calculated by subtracting the diameter of the **largest sphere that fits into the Object** from the intensity of the original channel.
If the option Background Subtraction is selected then the **Sphere Diameter** should be determined.

Sphere Diameter

The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of **Sphere Diameter** should be slightly larger than the diameter of the largest object expected to be found in the dataset.
If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

Detect Nuclei

Select this option and the Nucleus objects are included in the automatic creation process.

Source Channel

Select the respective source channel, from which the Nuclei are to be computed.

Smooth

Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.
Smoothing may require a great deal of memory if the dataset is very large. Uncheck the check box or use smaller ROI if your system does not have enough memory.
If the option Smooth is selected then the Filter Width value should be determined. Use either the automatically given value or type in a new value in the data field.

Filter Width

The value of the Filter Width determines the Gaussian filter width.
The value is either automatically calculated from the voxel sizes (oriented at the largest voxel dimension) or it can be directly entered into the numerical field. The value is calculated into image coordinates and not into fraction of voxels.

Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.
The width of the Gaussian filter is calculated by subtracting the diameter of the **largest sphere that fits into the Object** from the intensity of the original channel.
If the option Background Subtraction is selected then the **Sphere Diameter** should be determined.

Sphere Diameter

The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of **Sphere Diameter** should be slightly larger than the diameter of the largest object expected to be found in the dataset.
If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

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14.2.1.4 Threshold

Cell Threshold (Absolute Intensity)

In this creation step the Cell and Nucleus objects are detected and segmented.

Threshold

Activating/deactivating the Threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold. In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the threshold value is not activated (indicated by the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the threshold option is activated (indicated by the green button), the corresponding A/M button turns black.
As a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Manual
Define the requested threshold by clicking into the histogram or by entering the value directly in the data field.

The image's regions with intensity higher than the set threshold value are Cell objects to be created.

This option controls the visibility of the cell's objects and allows you an easier way to set the threshold parameters.
Click on the button and in the viewing area only Cell objects are displayed. The Nucleus and Vesicle objects are hidden from view.

Nucleus Threshold (Absolute Intensity)

Threshold

Activating/deactivating the Threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold. In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the threshold value is not activated (indicated by the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the threshold option is activated (indicated by the green button), the corresponding A/M button turns black.
As a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button

changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

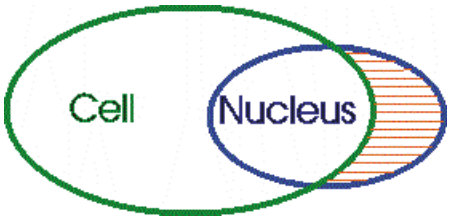
The image's regions with intensity higher than the set threshold value are Nucleus objects to be created.

Visibility of the Nucleus objects

This option controls the visibility of the cell's objects and allows you an easier way to set the threshold parameters.
Click on the button and in the viewing area only Nucleus objects are displayed. The Cell and Vesicle objects are hidden from view.

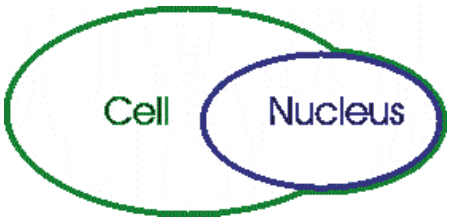
Cells Options

Cells options provide the user the possibility to refine the Cells and Nucleus objects segmentation. These options determine the segmentation result of the Nucleus voxel set detected outside the Cell (marked with orange lines).



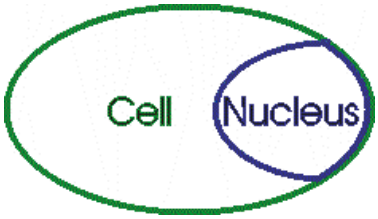
Expand Cell on Nucleus

This option defines the voxel set which is assigned, in the segmentation step, as a boundary of the Cell object. Areas of the image that have been initially segmented as Cells are expanded outside the original borders, to include a set of voxels which are defined in the segmentation step as Nuclei.
Resulting in a union between the set of voxels defining the Cells and the set of voxels initially defined as Nuclei.



Erase Nucleus outside Cell

This option defines the voxel set which is assigned as a boundary for the segmented Nucleus.
Any parts of Nuclei that are segmented outside the Cell object is removed from the preview area, leaving only set of voxel assigned as Nucleus within the Cell object. This means that the segmented Nuclei boundary will be reduced to the voxel set defined as the Cell object.



Enforce one Nucleus per Cell

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When the automatic algorithm does not properly segment the cells, creating cell objects containing two or more nuclei, this option can be used. When activated, this option performs a separation of cells and as a result of the process every single Cell contains only one Nucleus. The algorithm used for this separation is the **Marker Controlled Watershed**.

Fill holes in Cell and Nucleus

Under the Cell option several options for adjusting the current cell segmentation are provided.
If you have a Cell or Nucleus object containing many interior holes, use this command to eliminate the interior holes quickly, while still retaining the existing object segmentation.
The hole is defined as a voxel set that has an intensity less than the threshold value and which is completely and contiguously enclosed in all dimensions by the voxels assigned to either the Cell or Nucleus object.
If the option **Fill the holes in the Cell and Nucleus** are selected, holes inside the Cell and Nucleus object will be filled. The filling is accomplished by setting the voxel in each such set to a new intensity value and assigning them to the surrounding object.

After the threshold step, Vesicles detected outside of the Cell voxels set are purged.

Split Nuclei

This option supports the separation of two or more Nucleus objects that are in the Threshold step has identified as one.
The Split Nucleus method for segmentation in Imaris starts with defined seed points. You use Nucleus as seed points for the Nucleus segmentation that is performed in the next steps. During the process around each seed point, the region grows until a border stops the growing.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.5 Detect Nuclei Seed Points

Seed Detection

Estimated Diameter
Type in the estimated diameter of a Nucleus seed point.

14.2.1.6 Classify Nuclei Seed Points

The Seed Points filtering is incorporated into the creation process. This tab only appears, if the **Split Nucleus** option is checked.

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes

objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is

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visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.7 Classify Cells

The Cells objects filtering is incorporated into the creation process.

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.
Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated. Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

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Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.8 Classify Nuclei

The separate Nuclei objects filtering is incorporated into the creation process if you select the Cell Membrane algorithm setting.

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the

properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.
Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated. Clicking on the

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Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.9 Membrane Detection

The Membrane Detection algorithms set partition of a given image into two regions, one representing the objects to be detected and one representing the background.
All background regions are considered as a single region.
As the initial region for the object of interest, the detected Nuclei objects are used. First, the algorithm fits a

close border around the nucleus object region creating the initial size of the cell.
Then the algorithm is based on interactive boundary detection, where the border iteratively propagates until the optimal closed boundary is found by successively trying all possible positions in the object region.
The membrane detection process is should repeated by combining the algorithms and by using visual feedback until you are satisfied with the look of the segmented region.

Entirely smooth

This algorithm setting assumes that the intensities are statistically homogeneous in each region assigned to the image. The intensity of each region is approximated by a value of mean intensity.

Piece Wise smooth

In practice, large objects usually have a heterogeneous intensity distribution. The PieceWise smooth algorithm setting takes into account the presence of intensity variations. By using local region information, the algorithm divides the image region into sub regions with the intensities matching the local mean properties.

Remove Tiny Regions

If this option is selected the small complex regions are removed.

Diameter

The value stated in the Diameter field defines the maximum size of the region which can be considered for removal.

Max diameter

The value defines the maximum diameter of the region border to be created.
This parameter also affects the Maximum number of the algorithm iterations to be performed.

Shape

This setting affects the shape of the resulting border

Accurate/ Smooth

The setting of the Shape slider, is more to one side or the other, will influence whether the resulting border is smoother or more accurate than the original image characteristic.

Length /Curvature

The Curvature value constrains the shape of the resulting border. Setting the slider to be more on the Curvature side, results in a circularly shaped borderline.

Detect Button

Press this button to perform the next algorithm iteration.
The result of the algorithm calculation is visible in the viewing area.

Reset Button

When this button is selected, all cell membrane computation is reset to the initial state.
Please note: Intermediate cell membrane computation result are not saved.

Detection iterations number

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The value shows the number of the completed algorithm iterations.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.10 Detect Vesicles

Detect Vesicles

Select this option to start the automatic creation process for Vesicles.

The drop-down list shows the types of Vesicles that will be detected in the creation process.

To detect the additional vesicles types
To add a new Vesicles type, click on the button and choose the name. The new Vesicles type name appears in the list.

1. Click on the **Add New Type** Button
2. Enter a Vesicle name in the **Vesicles name** field
3. The new Vesicles type name appears in the list
4. Select the Source Channel
5. Define the Estimated Diameter
6. Decide on the Background Subtraction

Vesicle Name

This field allows you:

1. To enter the name for a newly added vesicle type or

2. To edit a name already defined

Source Channel

Select the respective source channel, from which the Vesicles are to be computed.

Estimated Diameter

Objects with size smaller than specified **Estimated Diameter** are not detected.

Background Subtraction

If checked, the background will be removed prior to Vesicle detection. Technically the background subtraction is used to smooth the image. The intensity equals the Gaussian filtered channel (Gaussian filtered by 3/4) minus the intensity of the original channel Gaussian filtered by 8/9 of sphere radius.

Add New Type Button
To add a new vesicles types, click on the button Add and choose the appropriate settings.

Delete Button
Select a Vesicles type in the list and click on this button to delete it.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.11 Classify Vesicles

The Vesicle filtering is incorporated into the creation process.

Click on the drop-down list and select the required Vesicle type to be classified.

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria.
First select the Vesicle type. Than select the filter type by selecting a variable from the drop-down list, and finally define the filter values, which will then limit the selection just to those objects that meet the set up criteria.
In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.
Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

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Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated. Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type

in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.12 Tracking

Tracking allows objects to be traced throughout sequential frames of a time dataset, so each object maintains a unique identity in the output measurements.
Tracking the Nucleus and Vesicle objects with respect to the inertial image coordinates is less significant than tracking their relative motion within the cell.
In order to cope with the spatial position of Nuclei and Vesicles within the Cell, the concepts of relative tracking are required.
The tracking of Nuclei and Vesicles objects is presented in relative terms of their patenting Cell objects, rather than their real coordinates on the screen.
Since the Cell object itself can be moving (relative to the inertial image reference frame), the tracking of Nuclei and Vesicle is **relative** to the parent Cell of a specified reference point.
The position of the Nuclei and Vesicle objects is specified as a relative distance from a reference point. This reference point is the Cell object center of mass.
The position of the individual Nuclei and Vesicles objects is calculated using two types of information. One is the absolute position of the selected object in terms of the inertial image reference coordinates and the other is the absolute position of the parent Cell object center. The **relative** Nuclei and Vesicles object's **position** is calculated by subtracting these two positional differences and is then tracked.
This concept of relative tracking allows direct measurements and statistical data comparison between moving cells to be calculated.

Track (Autoregressive Motion)

Track Nucleus

Select this option and the Nucleus objects will be tracked.

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Track Vesicles

Select this option and the Vesicles objects will be tracked.

Autoregressive Motion
This algorithm models the motion of each object as an autoregressive AR1 process. This model is appropriate if object perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user specifies the value of maximum distance. This value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the value of the maximum distance parameter, and because the AR1 algorithm performs correlations only to one previous time-point.

Parameters

Max Distance Cell
The value of the Max Distance specifies the maximum distance that a track Cell object could possibly move between two consecutive time points. This parameter disallows connections between a Cell and a candidate match if the distance between the predicted future position of the Cell and the candidate position exceeds the maximum distance.

Max Relative Distance Nucleus
The value of the Max Distance specifies the maximum distance that a track Nucleus could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

Max Relative Distance Vesicles
The value of the Max Distance specifies the maximum distance that a track Vesicles could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

Options

It can happens that the object segmentation fails for some time points. This can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Tracks if it reappears.

Close Gap
Select this option and the gap-closing algorithm is implemented.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size file.

The **Max Gap Size** defines the maximum number of the consecutive time points witch are allowed to be missing in order to a track to be continues.

Fill the gaps with all detected objects

If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

Cells Wizard Buttons

Button **Back**
With Back you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**
If you do not want to create Cells click on **Cancel**.

14.2.1.13 Classify Tracks

The Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Cells (over Time)** is checked (Algorithm).

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.
Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point,

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Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).

The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Tracks) is displayed at the bottom of the threshold histogram.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.2.1.14 Complete ROI

This tab only appears, if **Process entire Image finally** is checked (Algorithm).

Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Finish**
Click on **Finish** to end the calculation and display the results in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.2.2 Tab Settings

On the tab Settings you can choose the rendering methods and adjust the rendering quality to produce faster performance of the display.

Object Type

A choice of Object types is presented. For time series images the choice of the Object Type is in the drop down list. After choosing the Object type, a selection of the Setting Tab is listed.

Cell/Nuclei
Cell Tracks
Nuclei Tracks
Vesicle Type and Tracks

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14.2.2.1 Cell/Nuclei

Cell and Nuclei Style

Cell visible
This option shows or hides the Cell objects in the viewing area.
Nucleus visible
This option shows or hides the Nucleus objects in the viewing area.

Cell and Nuclei Style

Ortho Slicer
Ortho Slicer represents an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. Three orthoslicer planes show their intersection with a plane perpendicular to two of the coordinate axes
Change the slice direction if required by selecting the adequate plane.
The check buttons labeled as XY, XZ and YZ direction, are used to select the visibility of the corresponding slice.

The position of slices can be changed in the viewing area. Switch the pointer to the Select mode and then click on the plane and drag it to a new position.

Dataset visible (Slicers only)
This option is available only if orthoslicer options are selected. When this option is selected the dataset is shown on the slicers in addition to the Cell/Nucleus objects.

Oblique slicer
The Oblique Slicer represents a plane that can be freely moved and rotated within the data set and allows the user to look inside any object. The Oblique Slicer consists of a colored frame with a white double rod in the middle.
The position of the Oblique Slicer can be changed. Choose the pointer mode Select, highlight the Oblique Slicer in the viewing area and click on the bigger diameter rod. It will appear highlighted as it is selected, allowing you to move it and to set the Oblique Slicer to the required position.
The Oblique Slicer can be rotated around the x-, y-, and the z-axes. In the Select mode click the smaller diameter rod and it will be highlighted as it is selected. To rotate the Oblique Slicer, move the smaller rod.

MIP Rendering
This option visualizes the cells and nuclei as Maximum Intensity Projection (MIP). A Maximum Intensity Projection is a method for 3D data visualization. The MIP Rendering method uses the intensity value of the brightest voxel along the ray path traced from the viewpoint to the plane of projection.

Volume Rendering
This option performs a three-dimensional volume rendering and illustrates a 3D structure of the objects. Volume rendering enhances 3D structures compared to slice display and facilitates understanding of spatial relationship.

14.2.2.2 Cell/Nucleus Tracks

Cell/Nucleus/Vesicles Tracks

Tracks Style/Quality

The box Style/Quality has settings that determine the display of the connections between the Track objects.

Cylinder

Select this option to display the tracks connections as cylinders.

Line
Select this option to display the tracks connections as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter/Render quality
Depending on the previous selection you can either adjust the width of the line or the diameter of the cylinder.

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color, click on the Color symbol to open the Color Editor.

Radius Scale
An Edit field for the displacement vector radius.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points. The number of time points is determined with the slider position.

Slider
The Dragon Tail length can be adjusted using the slider. The slider determines the number of time points in the length of the Dragon Tail.

14.2.2.3 Vesicle Type and Tracks

Vesicles Style/Quality

On the tab Settings you can adjust the rendering style or quality.

Center Points
This option displays the Vesicles as center points.

Off
Select this option to disable the visualization of the Vesicles.

Sphere
Select this option to display the Vesicles as spheres.

Radius Scale box
The size of the Vesicles can be changed via the Radius Scale box.

The Render Quality
The Render Quality is adjustable using the slider. When the number of Vesicles is very large, adjust the Render Quality,to produce a faster display performance.

Tracks Style/Quality

The box Style/Quality has settings that determine the display of the connections between the Track objects.

Cylinder
Select this option to display the tracks connections as cylinders.

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Line
Select this option to display the tracks connections as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter/Render quality
Depending on the previous selection you can either adjust the width of the line or the diameter of the cylinder.

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color, click on the Color symbol to open the Color Editor.

Radius Scale
An Edit field for the displacement vector radius.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points. The number of time points is determined with the slider position.

Slider
The Dragon Tail length can be adjusted using the slider. The slider determines the number of time points in the length of the Dragon Tail.

14.2.3 Tab Creation

Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list, which step of the Cell Creation Wizard you want to re-enter.

For details please refer to chapter:
Menu Surpass - Cells - Algorithm
Menu Surpass - Cells – Tracking

Rebuild Objects Button
Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so that the Cell computation can be repeated for the same region, a different region, or the entire image. The Creation Parameters are listed as the table.

Remember Parameters Button

The Remember Parameters button allows you to save the complete set of the creation parameters and the relevant settings shown in the list.
Storing the parameters allows them to be easily reused, modified, or exchanged.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

Click on the Remember Parameters Button opens the Remember Creation Parameter window.

Remember Creation Parameter

Enter a name for the Creation Parameter set. This name identifies the Creation Parameter item in the Creation Parameter list used in the Edit/Preference/Creation_Parameters and would be used as default name in the subsequent creation wizard algorithms.

Add to Batch Button
The Add to Batch button places the command into the Batch coordinator with all the adjusted parameter values.

14.2.4 Tab Edit

Once the Cell creation wizard is completed, use these buttons for manual editing of the Cell objects. It allows you:

1. To import Surfaces or Spots and incorporate them in the Cells analysis.
2. To export objects from the Cells.
3. To clear the selection or delete all selected component of the cell object.

Import

Import Surface to Cell
To import the Surface as the Cell or the Nuclei Objects, click on this button. The window, **Import to Cell Object**, will appear.

Import Spots to Vesicles
To import the Spots as the Vesicles, click on this button. The window, **Import to Cell Object**, will appear.

Import to Cell Object window

Choose one of the objects from the drop-down list. Choose how the selected object type is to be imported and specify the selection and the time.
The Surface can be imported either as the Cell or the Nuclei.
The Spots can be imported only as the Vesicles.

Import as
Choose to import the object either as a new Vesicle type or as a previously detected Vesicle type.

To import the Spots object as an already segmented Vesicle type, please select the correct name from the drop-down list.

Selection
The user can decide either to import the **Selection** or the **All** objects of the specified component.

Time
The **time point** of the object selected can be imported either as the **Current time point** or the **All time points** .

Export

Selection
The **Selection** checkbox is used to export only the currently selected object.

All Timepoints

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The **All Timepoints** checkbox is used to export the object for all time points.

Export Cells to Surface
With a click on this button you export the Cell objects to a Surface object. The Surpass Tree list is automatically updated with the new item with the automatically generated name **Cells n Cell Export**. The exported objects can be then manually edited.

Export Nuclei to Surface
With a click on this button you export the Nuclei objects to a Surface object. The Surpass Tree list is automatically updated with the new item with the automatically generated name **Cells n Nucleus Export**. The exported objects can be then manually edited.

Export Vesicles to Spots
With a click on this button you export the Vesicles to a Spots object. The Surpass Tree list is automatically updated with the new item with the automatically generated name **Cells n Vesicles Export**. The exported objects can be then manually edited.

Full Export
With a click on this button you export all the Cell objects. The **Cell** and **Nuclei** objects are exported **to Surface**, while the **Vesicles** objects are exported **to Spots**.
The Surpass Tree list is updated and three new surpass objects are generated (two Surface items and one Spot item) with the automatically generated names **Cells n object-name Export**. Pressing this button has the same effect as pressing the three buttons above.

Process Selection

Clear Button
Click this button to clear the current object selection.

Delete Button
Click on this button to delete all the currently selected components.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.2.5 Tab Edit Tracks

Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.
The first row of the track-editing table displays the timeline. Each number represents a time point in the series.
The second row of the track-editing table displays the **object name**.
Multiple object types can be tracked easily since every object type appears in different colors.

Object ID

If the mouse pointer is on the object, a tool tip appears that identifies the object ID. The ID numbers of the objects are sequentially ordered column-wise from the top to the bottom of the table. The object maintains its unique ID number independent of the track being allocated to.
If the mouse pointer is on the connection, a tool tip appears that identifies the track ID number.

Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding

track (row in table) is highlighted.
In addition, the Statistical and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
For multiple selections press Ctrl+click (Mac: Command+click).

Visualization options

Zoom
Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.
Using the mouse wheel will have the same affect.
Pan
To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

Time indicator
The first row of the track-editing table displays the timeline. Each number represents a time point in the series. The current time point is indicated by vertical line highlighted with in current selection color.

To modify the time point, do one of the following:
1. Moving the slider in the Time bar modifies its position in time and changes the time point in the track editor.
2. Clicking on the time point within the track-editing table automatically moves the time line indicator to the corresponding point.
Double clicking on the time indicator, fixes the position of the current time point at the center of the track-editing table, regardless of the selected zoom or pan options)

Editing

Track Editor has several features that help you modify your tracks

Add tracks

To set the starting point of a new connection first you have to select the object.
Shift+left-click followed by a mouse movement on the object creates the new connection.
To establish a connection, drag the edge to the target object. If valid, the target object is highlighted (red).
Release the mouse button and the line segment clips automatically onto the new object, creating a new connection between the two objects.

Please note: The new connection is only possible between the objects of the same type. If you release the mouse button without being on a target object, the edge will disappear and a new connection will not be established.

A connection between objects of the same time point is also invalid.

Delete Tracks Edges

Tracks
Select the track and press backspace to delete the whole track.

Connections
To delete the connection, select the connection and press shift-click. The selected connection is deleted.

The process of deleting the connection splits the track. The first track part remains with the same ID number and stops at the time point of the deletion. The second part consists of the remaining deleted objects and the connections between them. It is automatically shifted to the bottom of the track-editing table and assigned a new track ID number.

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Connect unconnected components

Manually added, or previously untracked objects are displayed as separated circles. The procedure of creating new connections and connecting the objects is unchanged.

Show selection (working set)

This option allows you to work on subset of tracks.

Connect Track Button

Click this button to connect two existing tracks. Select two Spots in two different tracks at the subsequent time points (just one Spot per time point) and click on the button Connect Track. The connection is automatically created between all selected objects.

Disconnect Track Button

Select a object and click on the button Disconnect Track. The connection from the selected object to the next object in time sequence is deleted.

Correct Drift

If you have an object drift (e.g. a cell drift) during the image acquisition you can use the Correct Drift button to correct the drift for the whole data set.

The ImarisTrack tracks all objects in an image, even the objects that should be stationary.
The Correct Drift function gives the user the opportunity:

1) to specify the tracks related to the stationary objects
2) to assign these objects as stationary objects and
3) to adjust the position of the rest of the image objects based on this selection.

As a result of drift correction, the entire image is transformed in such a way that the selected objects remain stationary and within the new image each object's position (changes) based on the correct draft algorithm. Within the new image, the true movement of the objects can be then detected relative to the stationary objects.

In the accordance with the selected for **Result DataSets** options. the new image coordinates are calculated and displayed.

To make the Drift Correction on the data set the following steps are completed:

- Select the tracks associated with the stationary objects
- Select the algorithm for drift correction
- Select the option for the Result data sets
- Verify the selection and click on the OK button.

Please note: The drift correction changes the data set.

Based on the provided the track sub-selection the algorithm determines the new objects position for all time points.
If the tracks are selected, the algorithm will be performed on the sub selection. Otherwise, every track of the image is considered for calculations.

Algorithms

The Drift Correction is calculated based on the two algorithms, Translation and translation and rotation.

Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

Please note: The number of the selected tracks affects the algorithm calculation.

Selection of a single track gives a complete description of a translation.
Selection of a two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).
A better fit between the time points is achieved when three or more tracks are selected.

Result DataSet size

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.
Select the Result data set option to determine which new image part will be displayed.

Crop largest common region

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

New size equal to current size
The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

Include entire result

The final image will be the size of the combined area of the original and corrected image.
Selecting the appropriate Result dataset size may speed up the data visualization. This is especially important with (with large data sets.)

Correct spots positions

Activate this parameter and the spots are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.2.6 Tab Filter

Please note: Before using you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the

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properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray M A indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold and to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality)and the number and percentage of objects that fall into the particular filtering class are displayed at the bottom of the threshold histogram.

Duplicate Selection to New Surfaces Button
Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

14.2.7 Tab Statistics

Cells statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Cells. The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.
Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:
a) Overall
b) Detailed
c) Selection
In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.
To sort the columns click on the arrow in column heading. Click again to reverse the sort.

Overall Tab

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Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Detailed Tab

Tables in the Detailed Tab are showing a statistical data collected from all of the objects within the image. A pull down list offers three types of **Detailed** statistical calculations:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.
Object Statistical Annotation option is available for Specific values parameters.

Average values
The Average table contains values determined on the basis of all values within a set of the selected variable.
Columns are labeled as follows:
Min (Minimum)- the smallest value within a set of values of the selected variable
Max (Maximum)- the largest value within a set of values of the selected variable
Mean – value of the sum of all values divided by number of values within a set
Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables
Sum- the sum of all values within a variable set
Count- number of values within a set
Unit- variable unit
Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Statistical variable classification:

1. Statistical variables associated with objects (Cells, Nucleus, Vesicles -volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables the Average values are displayed per time point. Changing the time points, statistical variable values and images are consequently changed.
2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement length, track intensity) are not time dependent.

All values
If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.
Object Statistical Annotation option is available for Specific values parameters.

Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).
A pull down list offers three types of statistical calculations from a Selection:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.
Object Statistical Annotation option is available for Specific values parameters.

Average values
The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

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The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values
If the option All values is selected, all statistical values will be displayed for the selected image element.
Object Statistical Annotation option is available for Specific values parameters.

Buttons Overview

	PC:	Mac:
Select Cells	Left-click	Click
Select multiple Cells	Ctrl + left-click	Command + click

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.
Click on the button **Settings** and you switch to the window Preferences - Statistics - Cells.

Button **Search** - Search by object ID.
Type in the object ID in the data field. Click on the button **Search**.
- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Cells** - Duplicate Selection to new Cells.
Click on this button and all selected elements are copied to a new Cell. The new Cell appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation**
Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** or **All Values** are selected

Button **Export** - Export Statistics on Tab Display to File.
Only the statistics that are shown within the current tab will be exported.

Button **Export** - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.
All of the statistics (that are enabled in the statistics settings) will be exported.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:
Menu Edit - Preferences... - Statistics - Cells

14.2.7.1 Statistical Annotations

Statistical annotations provides the opportunity to view and annotate image objects with the statistical data values. Using this function, the individual objects in an image are labeled with the specified statistical values and the statistical data is displayed directly in the viewing area
Imaris objects can be labeled with a set of any number of the user-defined statistical values.
The Statistical Annotation button provides a convenient interface to label the object with the statistical data. Click this button and an object statistical annotation will be automatically generated in the viewing area. The selected object is annotated for all time points with the specified statistical values.

How to add Statistical Annotation

In the Statistical tab choose the **Detailed** or **Selection** Tab.
In the viewing area select the object or group of objects. Change to the pointer mode Select and click on an image object that has been detected/segmented. For multiple selections press Ctrl+click (Mac: Command-click)
In the **Detailed** or **Selection Tab** a pull down list offers **Specific Values** or **All values** as types of statistical parameters.
Select the statistical value associated with the particular object type you are working with.
Press the Statistical Annotation button .
In the viewing area the object label appears showing selected statistical values as a statistical annotation.

The statistical annotation shows by default the name of the selected statistical parameter and its value.
The color white displays the text of annotations associated with statistical values.

For a single object, all selected statistical parameters are placed in the same annotation box. Once the object is labeled, selecting the additional statistical parameter updates the object statistical annotation; the new parameter and its value is added in the viewing area.
If the image is a time series, the statistical annotation will be visible in the viewing area for all time points. Selecting a statistical variable that changes over time creates an statistical annotation that consequently changes with every time point.
The statistical annotation displays values always for the current time point.

The InPress options will allow you to modify and specify the contents and format of the object labels.
To modify and adjust the statistical label click on the Icon InPress , press Ctrl + Shift + P, or in the menu bar select File – InPress.

14.2.8 Tab Color

Choice of the Object type is presented in a drop down window. Select Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle (multiple types) or Vesicle Tracks.
Depending on the chosen object type selection of the variables is listed.

The tree color types are available:
Base
Statistics Coded
Time Mapped

14.2.8.1 Tab Color-Base

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

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Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.2.8.2 Tab Color-Statistics Coded

Depending on the chosen object type the Statistical variable selection is listed in the drop down window. For example, if Cell/Nuclei/Vesicle is chosen as the Object type, available Statistical data will be the Cell/Nuclei/ Vesicle linked variable. Similarly, variables for Tracks will be listed, if the object type is selected to be Tracks.

Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area. The upper one is the colorbar range for Cel/Nucleus/ Vesicles and lower one is the colorbar for the Tracks.

Show Colorbar
Check the box to see the statistics colorbar in the viewing area.

Show Title
Check this box to see the title in the statistics colorbar.

Show Range
Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**
Click the **Reset** button to set the image back to the original values.

Button **Load**...
Click on the button **Load** to display the Import Color Table File color window. Enter the path and the requested File name and/or File type and click Open. The selected file is loaded and displayed.

Colormap range

Colormap range-specify the limits for the colorbar.
Min - lower color limit
Max - upper color limit
Button **Auto**

Click the button **Auto** and the system detects the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

14.2.8.3 Tab Color-Time Mapped

This option is only available when Tracks (see above) is selected as an Object Type. The color range for the Tracks goes from blue to white to the range of time points.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

14.3 Clipping Plane

The Clipping Plane cuts away objects on one side of the plane. It can be freely moved and rotated in the scene and allows you to look inside any object. The Clipping Plane consists of a colored frame with a white double rod in the middle.
To change the position and orientation of the Clapping Plane in the View Area the Pointer must be in Select mode.

How to Add a new Clipping Plane?

- Select menu Surpass - Clipping Plane.

The Surpass Tree list is automatically updated and in the Clipping Plane - Properties the Settings parameter are displayed. The Clipping Plane is displayed in the viewing area.

To clip another object, this object must be placed behind the clipping plane within the Surpass Tree. It must be in the same group as the Clipping Plane and it must be placed below the Clipping Plane.

Change Position of Clipping Plane

The position of the Clipping Plane can be changed. Choose the pointer mode Select, highlight the Clipping Plane in the viewing area and click on the bigger diameter rod. It will turn colored as it is selected, move it to set the Clipping Plane to the required position.

Rotate Clipping Plane around Axes

The Clipping Plane can be rotated around the x-, y-, and the z-axes. In Select mode click the smaller diameter rod. It will turn colored as it is selected, move it to rotate the Clipping Plane.

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

14.3.1 Tab Settings

Position

Button **Recenter**
The Clipping Plane center is moved to the data set center.

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Orientation

Button **YZ Plane**
Button **XZ Plane**
Button **XY Plane**
The Clipping Plane is oriented parallel to either the yz-plane, the xz-plane or the xy-plane.

Button **Flip**
Click on the **Flip** button to flip the orientation of the clipping plane.

Configuration

Button **Store**
Button **Recall**
After changing the position and/or orientation of the Clipping Plane, the new configuration can be stored by clicking on the **Store** button. The position previously stored can be recalled by selecting the **Recall** button.

Camera

Button **Orthogonal View**
The camera is set to a position which is perpendicular to the Clipping Plane.

Show Frame
To make the frame visible, check this box.

Perpendicular Movement

The pointer must be in the pointer mode Select. Place the mouse within the image. To change the orientation of the rod press the Ctrl button. The Clipping Plane is moved in the directions parallel to the plane.

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

14.3.2 Mouse & Keyboard PC

Clipping Plane Mouse & Keyboard Functions

Left-click on plane & drag	Move plane to desired position
Left-click on the bigger diameter rod & drag	Move plane to desired position
Left-click on the smaller diameter rod & drag	Rotate plane to desired position

See also:
Addendum - Mouse & Keyboard PC

14.3.3 Mouse & Keyboard Mac

Clipping Plane Mouse & Keyboard Functions

Click on plane & drag	Move plane to desired position
Click on the bigger diameter rod & drag	Move plane to desired position
Click on the smaller diameter rod & drag	Rotate plane to desired position

See also:
Addendum - Mouse & Keyboard Mac

14.4 External Object

Surpass provides the ability to import External objects (both, non-Imaris and Imaris objects) and display them concurrently with a Surpass object for comparison.

Only the External objects with the extension ***.iv** (Inventor file) or with extension ***.vrml** (VRML2 files) can be imported.

The imported External objects can be rotated, scaled and translated.

For example, previously calculated Surfaces, which have been exported as Inventor files, can be imported to another data set.

How to Add a new External Object?

- Select menu Surpass - External Object.

The Surpass Tree list is automatically updated and in the External object - Properties the Settings parameter are displayed.

See also:
Menu Surpass - Export Selected Objects ...

14.4.1 Tab Settings

The object properties area displays a number of parameters for the positioning and display of the External object. The following display/handling options can be checked:

Box Handle
The object shows a surrounding box with handles. These handles allow to transform/distort the object in the x-, y-, and z-direction.

Trackball
The object shows surrounding circular lines. These lines allow rotation of the External object in any direction.

File Path Field
Display of the file path (to load the object, see below).

Button **Load...**
To select and load a data set as a External object click on the button **Load...** and the Load External Object File window is displayed. Select the file to be loaded as External object. The External object will be only loaded if the selected files have the extension ***.iv** (Inventor file) or ***.vrml** (VRML2 files). Click on the button Open.

Button **Reset Size and Location**
Clicking this button sets the External object back to its original size and position before any translational, scaling or rotational operations have been performed.

Fix Ratio
If this option is checked and if scaling is performed on the External object by entering a value, the same scaling factor is applied to all three dimensions.

Position/Scaling

The following positional/scaling options are available:

Translation

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Use these fields to move the External object in the x-, y-, or z-direction.

Rotation
Use this field to rotate the External object around the x-, y-, or z-axis.

Scaling
Use this field to scale the External object in one of the dimensions (or all together if Fix Ratio, see above, is checked). Fix Ratio is only valid with this method of scaling.

See also:
Menu Surpass - Export Selected Objects ...

14.4.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.4.3 Mouse & Keyboard PC

External Object Mouse & Keyboard Functions

Left-click white box & drag	Scale object from center
Left-click white box + Ctrl & drag	Scale object from opposite corner
Left-click trackball & drag	Rotate object
Shift + left-click trackball & drag	Set up a rotation axis

See also:

Addendum - Mouse & Keyboard PC

14.4.4 Mouse & Keyboard Mac

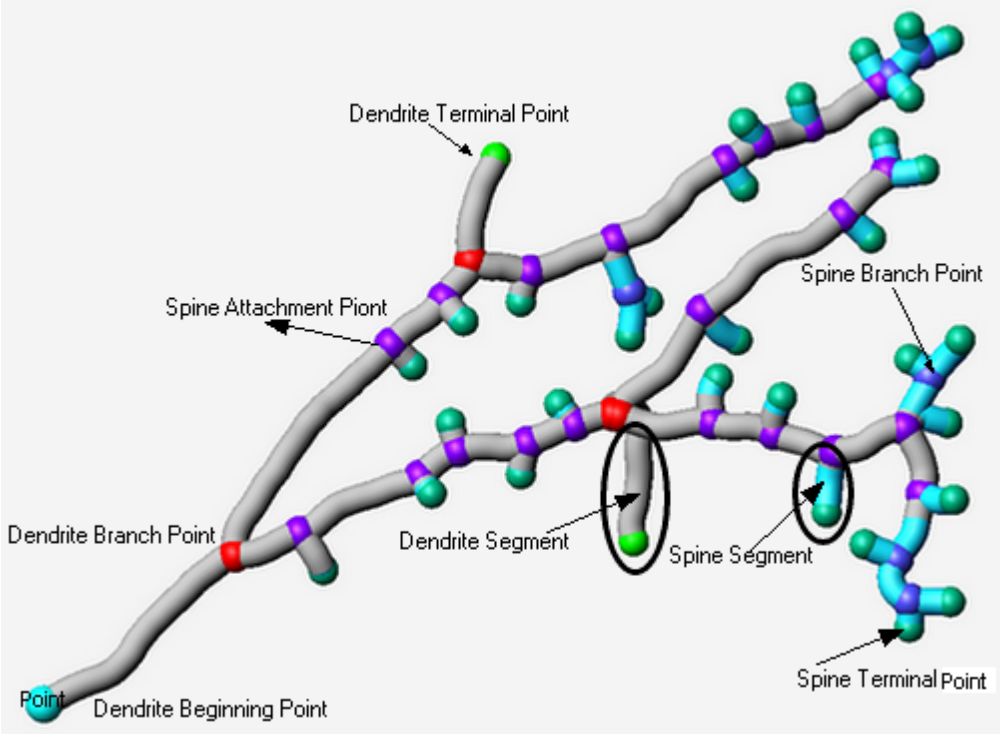
External Object Mouse & Keyboard Functions	
Click white box & drag	Scale object from center
Click white box + Ctrl & drag	Scale object from opposite corner
Click trackball & drag	Rotate object
Shift + click trackball & drag	Set up a rotation axis

See also:
Addendum - Mouse & Keyboard Mac

14.5 Filament

General

In IMARIS the dendritic trees are represented by a set of **vertices** and **edges**. The **Vertex** is drawn as a node or a dot and defines the center axis of the dendrites. An **edge** is presented as a line connecting two vertices. The entity of vertices and edges is referred to as filament graph.
Parts of the filament graph can be classified as spines either collectively, based on some statistical values, or individually by the user, by determined specifications.
Starting, ending and branching vertices are classified into **dendrite beginning point, dendrite branch point, dendrite terminal point, spine attachment point, spine branch point, and spine terminal point** depending on their position in the graph (refer to the picture below).
Segments are defined as the collection of edges between two starting, ending, or branching vertices. Segments are classified either as **dendrite segments** or **spine segments**. In a dendrite the edge is called a **dendrite edge**, in a spine it is called a **spine edge**.



The Imaris FilamentTracer offers features to segment, track, edit, draw, display, measure and annotate

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Filaments. The FilamentTracer processes one channel (color) at a time and creates objects that qualify as Filaments (e.g length and average thickness comply with criteria).

After an automatic Filament creation (see Creation Wizard) you can launch an editor operation to delete Filaments, connect Filaments, or draw new Filaments. You may also skip the automatic Filament generation and process the data with semi-automatic or manual segmentation and Filament generation (see the Tab Draw).

Generated Filament objects will be visualized in the context of Surpass. This will allow multiple viewing combinations in which the Filaments are displayed individually or together with other cellular components that may have been imaged in other channels.

Properties and measures of entire filament graph or the selected parts (Points, Edges, Dendrite, and Spines) are automatically generated in the Tab Statistics and it can be exported in tabular format or directly to MS Excel.

How to Add a new Filament?

- Select menu Surpass - Filament.

The Surpass Tree list is automatically updated and you start in the first window of the Creation Wizard (see below).

See also:
Surpass View - Filament - Creation Wizard (for automatic Filament creation)
Surpass View - Filament - Tab Draw (for interactive Filament tracing method)

14.5.1 Data Input

The FilamentTracer is designed to operate on 3D time-resolved images. These images can be multi-channel (multiple colors) but only one channel will be traced at a time. The following input images are acceptable:

- The ideal input is a fluorescently labeled confocal image. Such images generally have good contrast (selective labeling) and good resolution in all three spatial dimensions.
- 3D images can also be acquired using a standard microscope (non-confocal) equipped with a CCD camera and a stepper motor. Non-confocal optical arrangements do not provide resolution along the optical axis which is why every single plane consists of in-focus components and out-of-focus components. These images require deconvolution prior to tracing. The technique of deconvolution uses information about the imaging process (the point spread function) to remove the out-of-focus components from every section.
- Finally there are 3D images taken in transmission light microscopy. Staining is usually less specific than in the fluorescent case and it will be more difficult to automatically segment the Filament. Deconvolution can also be applied to transmission images although - theoretically - its performance is weaker than in the fluorescent case.

14.5.2 Creation Wizard

The automatic filament graph compilation (creation) is guided by a wizard-like user interface and consists essentially of five steps.

- Select the appropriate algorithm (or quit the automatic filament creation and draw the filaments interactively).
- Define a Region of Interest
- Select source channel and do some pre-processing.
- Segmentation to specify filamentous and non filamentous parts.
- Graph compilation and finishing.

The Creation Wizard allows to go forth and back until the optimal parameters are found.
In the first window of the Filament Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.
The most convenient way to apply the same creation parameters to different data sets is to use the **Load Parameters** option.

See also:
Surpass View - Filament - Creation Wizard - Algorithm
Surpass View - Filament - Creation Wizard - Preprocessing
Surpass View - Filament - Tab Draw (for interactive filament tracing method)

14.5.2.1 Algorithm

In this first window of the Creation Wizard you decide, if you want to go for an fully automated filament creation using one of the two predefined algorithms (AutoPath-no loops or Threshold-loops, see below); or if you want to draw the filaments interactively.
If you do not want to use the automatic filament creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the interactive filament tracing method.

Load Parameters

This option lets you use the identical creation parameters for multiple datasets.
The option to Load Parameters is only available if the set of the creation parameters has already been saved under the Creation tab using the Remember Parameters button.
Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Load Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

If you do not want to use the automatic Cell creation wizard select the **Skip automatic creation, edit manually** button. If you do not want to use the automatic Cells creation wizard select the **Skip automatic creation, edit manually** button.

Select Algorithm

The automatic filament creation provides two algorithms to choose from:
AutoPath (no loops)
Threshold (loops)

AutoPath (no loops)
The automated AutoPath Algorithm:
- **Produces a tree-like filament**
- **Is based on local intensity contrast**
- **Connects large start- and small end-points**

If you select this algorithm please refer to chapter: Surpass View - Filament - Creation Wizard - Autopath Algorithm (no loops)

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Threshold (loops)
The Threshold based Algorithm:
- **Produces a filament with loops**
- **Is based on an absolute intensity threshold**
- **Thinning to a skeleton**

If you select this algorithm please refer to chapter Surpass View - Filament - Creation Wizard - Threshold Algorithm (loops)

Calculate Diameter of Filament from Image

Check this box and the Filament diameter is calculated based on the image data.
Uncheck the box and the Filament diameter will taper smoothly from the starting point to the end points.

Segment a Region of Interest-ROI

A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.

Track (over time)

Check this box if you want to track Filament over time, for details please refer to the tab Tracking.

Filament Wizard Buttons

Button **Next**
Press **Next** to proceed with the next of the Creation Wizard.

Button **Finish**
Press **Finish** and based on the selected algorithm (see above) Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

Button **Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

14.5.2.2 Autopath Algorithm (no loops)

The Autopath (no loops) algorithm consists of the following steps:

Region of Interest
Dendrite Points Diameter
Classify Dendrite Points
Dendrite Diameter
Spine Points Diameter
Classify Spine Points
Spine Diameter
Tracking
Finish

14.5.2.3 ROI

Region of Interest 1

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

X, Y, Z, Time
Min, Max, Size
Minimum, maximum and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields. Alternatively the location and size of the ROI can be adjusted directly in the viewing area.
Size of the ROI:
1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X ,Y and Z.
Location of the ROI:
1.Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.
The numbers in the value fields are updated automatically.

Memory Usage:
Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button **Add**
Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button **Delete**
Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

Total Memory Usage:
Display of the memory usage for the image size within the ROI.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create Filament automatically click on **Cancel**.

14.5.2.4 Dendrite Points Diameter

Select Source Channel

Select the respective source channel, from which the Dendrite structure is to be created.

Starting Points

Detect a new starting point -a new starting point of the filament will be determined, based on the value given for the largest diameter.

(largest Diameter: e.g. Dendrite Beginning)

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Enter a value of the maximum (largest) diameter of dendrite to specify the Starting point diameter.

Seed Points

(Thinnest Diameter e.g. Dendrite Ending)
Enter a value of the minimum (smallest) diameter of dendrite to specify the Seed points diameter.

Rebuild Options

As part of the rebuilding process in step three of the Creation wizard – Dendrite Point Diameter, a new option exists for selecting a **Starting Point**.

Use Existing Dendrite

Use Existing Dendrite- the previously determined dendrite points will be used in the creation of new starting point. Therefore, in the next creation step (Starting point threshold) an option for Manual Start point editing in (shift+right click) will not be available.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Press **Finish** and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

Button **Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

By reentering the Creation Wizard, either after selecting Rebuilding Algorithm or for Processing additional ROI (s) a new option exists for selecting a Starting Point.

See also:
Surpass View - Filament - Tab Settings
Surpass View - Filament - Tab Draw (for interactive filament tracing method)

14.5.2.5 Classify Dendrite Points

Thresholds are used to classify the detected dendrite's starting points and seed points.
The detected dendrite starting point(s) are visualized in the image by different colors. The respective histograms are displayed.
The threshold can be set manually or automatically.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Starting Points Threshold

Display of the starting points threshold and the respective histogram. The threshold can be adjusted by entering a new value in the data field or by moving the colored threshold line (click & drag) in the histogram. The updated points are visualized in the viewing area.

Manual Starting Points addition/deletion in Creation Wizard

Manual addition of starting points in the filament structure is available by pressing Shift+ right-click. Shift+ right-click on an existing filament start point (created either automatically or manually added) and the selected starting point will be deleted.

Seed Points Threshold

Display of the end points threshold and the respective histogram. The threshold can be adjusted by entering a new value in the data field or by moving the colored threshold line (click & drag) in the histogram. The updated points will be visualized in the viewing area.

Manual Seed Points Addition/Deletion in Creation Wizard

Manual Addition of Seed points in the filament structure is available by Shift+ left-click. Shift+ left-click on an existing point, (created either automatically or manually added) and the selected point will be deleted.

Remove Seed Points Around Starting Point(s)

Check the box and all seed points located within the radius of the sphere centred at the starting point are removed. The boundary of the sphere is defined by the value Diameter of Sphere Region(s).

Diameter of Sphere Region(s)
Enter the diameter of the sphere centred at the beginning point. Default value set to twofold of the starting points diameter.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

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Button Cancel
If you do not want to create a Filament automatically click on **Cancel**.

14.5.2.6 Dendrite Diameter

The option is only available if in the step Algorithm the Calculate Diameter form the image is selected.
The threshold value is based on the local contrast and the respective histogram is displayed.
The defined threshold value is used for the dendrite diameter calculation.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

The effect of changing the threshold value is directly visible in the viewing area.

Algorithm

The Algorithm provides two methods to calculate dendrite diameter.

Shortest distance from distance map
The dendrite diameter is calculated as the shortest distance between dendrite center point and dendrite surface.

Approximate circle of cross section area
The diameter of the approximate circle of cross-sectional area is determined as a value of the dendrite diameter.

Filament Wizard Buttons

Button Back
With **Back** you return to the previous window of the Creation Wizard.

Button Next
Press **Next** to proceed with the next window of the Creation Wizard.

Button Finish

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create a Filament automatically click on **Cancel**.

14.5.2.7 Spine Points Diameter

Spine

Detect Spines
Check this box to Optimize the Creation wizard for Spine Detection; creating the highly branched filament structure.
Uncheck the box Detect Spines and the Spine channel selection and the Seed Points diameter are disabled. Spines detection and creation are not performed in the following creation procedure.

Select Source Channel

Select the respective source channel, from which the spines are to be computed. The default channel is the same as for the dendrite detection.

Seed Points

(Thinnest Diameter e.g. Small Spine Head)
Enter a value of the minimum (smallest) diameter of spine head to specify the Seed points diameter.

Maximum Length

The Maximum Length is estimated spine length, the length from the dendrite center to the furthest Spine seed point.

Allow Branch Spines
Check the **Allow Branch Spines** option to allow formation of the spines's bifurcation points. To form bifurcation point the two spine's vertices have to be in the vicinity of 1 voxel.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create a Filament automatically click on **Cancel.**

14.5.2.8 Classify Spine Points

The option is only available if in the step Points Diameter the Detect Spine option is selected.

Spine

Seed Points Threshold

Thresholds is used to classify the spines seed points. The detected Spine seed points are visualized in the image. The respective histogram is displayed.

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The threshold can be set manually or automatically.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

The threshold can be adjusted by entering a new value in the data field or by moving the colored threshold line (click & drag) in the histogram. The updated points are visualized in the viewing area.
The effect of changing the threshold value is directly visible in the viewing area.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create a Filament automatically click on **Cancel**.

14.5.2.9 Spine Diameter

The option is only available if in the step Points Diameter the Detect Spine option is selected.
The threshold value is based on the local contrast and the respective histogram is displayed.
The defined threshold value is used for the Spine diameter calculation.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

The effect of changing the threshold value is directly visible in the viewing area.

Algorithm

The Algorithm provides two methods to calculate Spine diameter.

Shortest Distance From Distance Map
The Spine diameter is calculated as the shortest distance between spine center point and spine surface.

Approximate Circle of Cross Section Area
The diameter of the approximate circle of cross-sectional area is determined as a value of the spine diameter.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create a Filament automatically click on **Cancel**.

14.5.2.10 Tracking

The filament object consists of a set of the dendrites, the spine points and the connection between them. Tracking allows objects to be traced throughout sequential frames of time dataset. Each object maintains an unique identity in the output measurements.

This tab only appears, if the Track (over Time) is checked (Algorithm Settings).

The Filament tracking tab provides Autoregressive Motion algorithms, which model the filament movement.
The algorithm utilizes the object's direction and speed information from one single time point to predict the best potential object position at the next time point.
Based on the tracking of the entire Filament graph, the algorithm predicts the future positions of the Dendrite Beginning Point, Branching and Terminal Points as well as the Spine Attachments, Spine Terminal Points Based on these predicted positions, the best matches to the candidate points can be determined. The algorithms use a Linear Assignment algorithm and a Total Cost function to find the best track connections. The total cost computation is based on the change in distance.

Autoregressive Motion

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This algorithm models the motion of the filament graph's center of gravity as an autoregressive AR1 process. The AR1 model looks back to previous time-point, and predicts that the filament object will move again the same distance and in the same direction. The user-specified maximum distance value is the distance in which object is allowed to deviate from the predicted position. Some changes in direction are tolerated depending on the setting of the Max. Dist. parameter. and because the AR1algoritham calculates the position based on only one time-point.

Track Filaments Center of Gravity

Max Distance

The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.
It can happen that the object segmentation fails for some time points, which can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Tracks if they reappear.

Close Gap

Select this option and the gap-closing algorithm will be implemented.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size file.

The **Max Gap Size** defines the maximum number of the consecutive time points witch are allowed to be missing in order to a track to be continues.

Track Dendrites

Select this option and the Dendrite points will be tracked.
When the Track Dendrite option is not selected the drop-down list remains inactive.

From the drop-down list you can select the type of Dendrite points to be tracked. There are two options:

- Dendrite Branch Points
- Dendrite Terminal Points

Please note: The tracking of the Dendrite Beginning point is automatically computed based on the tracking of the entire Filament structure.

Enable

Check the box to enable the selected Dendrite points to be traced

Max Distance

The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.

It can happen that the object segmentation fails for some time points, which can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Tracks if they reappear.

Close Gap

Select this option and the gap-closing algorithm will be implemented.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size file.

The **Max Gap Size** defines the maximum number of the consecutive time points witch are allowed to be missing in order to a track to be continues.

Please note: The spine tracking options are only available if the spines have been previously created.

Track Spines

Select this option and the Spine points will be tracked.
From the drop down list select the type of Spine points to be tracked. There are three options:

- Spine Attachment Points
- Spine Branch Points
- Spine Terminal Points

Enable track

Check the box to enable the selected Spine points to be traced.

Close Gap

Select this option and the gap-closing algorithm will be implemented.

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size file.

The **Max Gap Size** defines the maximum number of the consecutive time points witch are allowed to be missing in order to a track to be continues.

Please note: The spine tracking options are only available if the spines have been previously created.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

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Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create a Filament automatically click on **Cancel**.

14.5.2.11 Finish ROI Processing

The selected parameters will be used to complete the computation of a Filament structure in an image. By selecting one of six finishing options a Filament structure will be created in the following ways:

1. **Additional Regions** - re-enters creation process (in step two-ROI) and uses the same set of parameters for a new ROI to create the filament structure.This option is only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for all time points with the same set of parameters.

2. **Entire Image in All Time Points-** the entire image is processed with the same parameters used for the ROI processing and the filament structure is created for the entire image and all time points. Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters.

3. **All Done**- creates a filament structure only within an ROI.

Please refer to Tab Creation. In step three of the Creation wizard – Point Diameter, an option exists for selecting a Starting Point (Use existing filament or Detect a new starting point).

Note: The Use Existing Filament option requires that the new ROI overlaps with some portion of the existing filament.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create a Filament automatically click on **Cancel**.

14.5.2.12 Threshold Algorithm (loops)

The threshold (loops) algorithm consists of the following steps:

- Preprocessing
- Segmentation
- Graph Compilation
- Finish

14.5.2.13 Preprocessing

Source Channel

Select the respective source channel, from which the Filament structure is to be computed.

Feature Preprocessing

Enable Feature Preprocessing
Check the box and the following parameters are adjustable. Uncheck the box and the following parameters are grayed. Preprocessing is not necessary if the data is already deconvolved or if the noise has already been removed by a Gaussian filter. The preprocessing will be applied to the source channel and the result will be added as new Filament Working channel in the Display Adjustments.

Appr. Filament Diameter
An approximate minimal Filament diameter has to be provided.

Preserve Edges
You can also enable an edge preserving preprocessing to maintain filamentous structures.

Before continuing you can inspect the result of the previous step with any viewer of Imaris: Slice, Section, Gallery, Easy3D, Surfaces etc.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Press **Finish** and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

Button **Cancel**
Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to chapter Surpass View - Filament - Tab Draw to adjust the parameters.

See also:
Menu Edit - Show Display Adjustment
Menu Image Processing - Thresholding - Connective Baseline
Surpass View - Filament - Tab Draw (for interactive filament tracing method)

14.5.2.14 Segmentation

Segmentation

Fill Cavities (fully enclosed volumes)
Check this box to avoid hollow regions. These regions would be enclosed by a Filament mesh, which is not desired in most of the cases.

Connective Baseline
Type in two values for the lower threshold (colored line) and upper threshold (red line). You can also click into the histogram. Left mouse button for lower, right mouse button for upper threshold or you can click & drag the respective line.

Simple thresholding methods classify voxels purely based on a single intensity value. This approach often does not lead to good results because the staining, the illumination, and the detection operate within a precision range. The connective thresholding scheme takes care of this and searches for object voxels using an intensity range rather than a single intensity.

You must provide two threshold values. The lower threshold (colored line) is the intensity below which a voxel is recognized as part of the background, the upper threshold (red line) is the minimal intensity for a voxel to be

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part of the Filament object. Voxels with an intensity value between the two thresholds are treated as part of the Filament object if and only if they are connected to a voxel within intensity above the upper threshold ("double thresholding" with "feature-AND").

The result of the segmentation process described above is a clean image where the non-zero voxels are describing the objects (Filaments and some non-filamentous structures). Because such a description of the data is not suitable for morphometric analysis the structures are converted. Graph compilation is a process involving several image processing operations such as skeletonization, erosion, dilation and the creation of a vector definition for the Filaments. The result of the process is a database (kept in memory) describing the Filaments as a set of segments that may contain branches.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

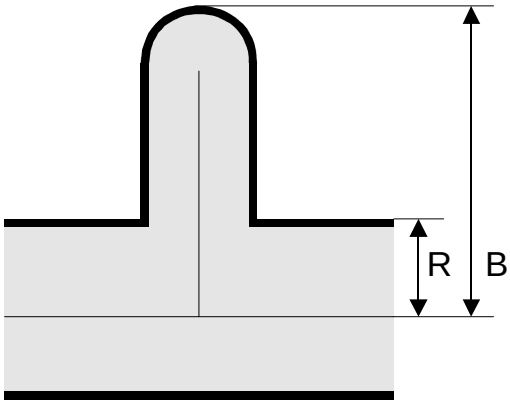
Button **Cancel**
If you do not want to create a Filament click on **Cancel**.

See also:
Menu Image Processing - Thresholding - Connective Baseline

14.5.2.15 Graph Compilation

Graph compilation

Minimal Ratio of Branch Length to Trunk Radius
Enter a minimal ratio of branch length to trunk radius (see figure below). Branches shorter than this value will be removed.



Ratio of branch length (B) to trunk radius (R).

Find Dendrite Beginning Point
A dendrite beginning point has to be specified for specific statistic values (e.g. tree order). The point is selected from the trunk position with the biggest radius.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**
If you do not want to create a Filament click on **Cancel**.

14.5.2.16 Finish

Finish

To validate the automatically generated graph and the prior segmentation, you can inspect the result together with numerous Surpass modules: Ortho Slicer, Volume, Surfaces etc. You can optionally superimpose the segments with the original slices of the image stack using Imaris OrthoSlicers. OrthoSlicers are planes, which are perpendicular to any of the three spatial axes and extend throughout the entire image volume.

Build Filaments for all Time Points
Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters. They will be visible within the Surpass Tree. However the 3D Filament graph is only visible according to its time point.

Delete Working Channel
The Filament Working channel can be kept for other use. Principally it is not needed any more when finishing the Filament graph.

Filament Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
This button is grayed in the last window of the Creation Wizard.

Button **Finish**
Click on **Finish** to end the calculation and display the results in the viewing area.

Button **Cancel**
If you do not want to create Filaments click on **Cancel**.

14.5.3 Filament Properties

When the creation process is finished, the tab Create disappears while some other property Tabs appear. They allow the manipulation of the filament graph:

Tab Create
Only appears when you Add a new Filament.

Tab Settings

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Change Filament appearance and inspect selection statistics.

Tab Creation
Re-enter the creation process.

Tab Draw
Manually draw filaments and connections.

Tab Edit
Select automatically filament parts and delete or smooth them.

Tab Statistics
Inspect all statistic values.

Tab Color
Change the color appearance (material) of the filament.

See also:
Surpass View - Filament (Add a new Filament)
Surpass View - Filament - Tab Settings
Surpass View - Filament - Tab Creation
Surpass View - Filament - Tab Draw
Surpass View - Filament - Tab Edit
Surpass View - Filament - Tab Statistics
Surpass View - Filament - Tab Color

14.5.4 Tab Settings

On the tab Settings you can edit and adjust the display options.
Select the Filament option to adjust quality and style of the filament graph.
Select the Filament Tracks option and you can edit and adjust settings that determine the display of connections between the Track filament graphs.
Select the Point Tracks option and you can edit and adjust settings that determine the display of connections between the **dendrite beginning, branch and terminal points, as well as spine attachment, branch and terminal points** of the filament graphs.

14.5.4.1 Filament

Style

Line
Cylinder
Cone
The visualization of the graph is done as lines with a specified thickness (pixel), as cylinders (with a defined diameter), or cones (using the measured graph diameter).

Show Dendrites

Check the box in the heading to highlight dendrite points in the filament. The special points are highlighted with color-coded spheres.

Beginning Point

Check this box to highlight the dendrite beginning points.

Branch Points
Check this box to highlight the dendrite branch points.

Terminal Points
Check this box to highlight the dendrite terminal points.

Show Spines

Check the box in the heading to highlight spine points in the filament. The special points are highlighted with color-coded spheres.

Attachment Points
Check this box to highlight the spine attachment points.

Branch Points
Check this box to highlight the spine branch points.

Terminal Points
Check this box to highlight the spine terminal points.

Render Quality

With the slider you can adjust the render quality of the filament. Moving the slider to the left reduces the render quality, moving it to the right increase render quality.

14.5.4.2 Filament Track

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Cylinder
This option displays the tracks connections dawn as cylinders.

Line
This option displays the tracks connections dawn as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter
Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality
When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Filament Tracks

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Radius Scale

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Edit field for the displacement vector radius.

Color Editor
Select a desired color for the displacement arrow.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider
Adjust the number of time points using the slider.

14.5.4.3 Point Tracks

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Cylinder
This option displays the tracks connections dawn as cylinders.

Line
This option displays the tracks connections dawn as lines.

Off
Select this option to disable the visualization of the Tracks.

Width
Diameter
Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality
When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Filament Tracks

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Radius Scale
Edit field for the displacement vector radius.

Color Editor
Select a desired color for the displacement arrow.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider
Adjust the number of time points using the slider.

14.5.5 Tab Creation

Rebuild

Imaris stores the list of operations and the mapping of parameters.

Click on drop down window and six choices are available for re-entering the Filament Creation Wizard.

Rebuild All
Rebuild Dendrite
Rebuild Dendrite Diameter
Rebuild Spine
Rebuild Spine Diameter
Rebuild Tracks

Select the rebuild algorithm to be applied from the list. As you select the rebuild algorithm, the creation wizard is re-entered in appropriate step.
The wizard retains all the prior parameters allowing you either to reused or edit them while re-creating the filament structure.

If you choose the rebuild option, the window Rebuild Diameter opens which performs the diameter calculation for either the Rebuild Dendrite Diameter or the Rebuild Spine Diameter.

Window Rebuild Diameter

Select the Specific Source channel, from which the Dendrite/Spine structure is to be computed. To specify the channel, enter the channel number in the field.

The Creation Wizard is using previous parameter settings as initial values. You can easily change just a few parameters without going in detail through all the parameters.

Spine Rebuilding

The spine rebuilding options are only available if in the spines are previously created.

The spines can be created at any later time by re-entering the creation wizard, since the spine detection and creation is the default last step of the Filament Creation Wizard.

Keep Data

The option **Keep Data** should be selected depending on whether the existing Filament graph should be **discarded**, or **re-used** in the Filament rebuild process.
If this option is selected, the previously created filament structure is kept. The Wizard creates a newly added filament that is merged with the existing one.
If the option is not selected, the previously created filament structure is discarded and the Wizard creates a new filament structure.

All Time Points

The option **All Time Points** should be selected depending on whether the parameters used for creating the Filament structure should be applied to the selected time point only or to all time points.
If this option is selected, the same set of creation parameters used for selected time point is reused to the create filament structure for all time points.
If the option is not selected, the creation parameters are used only for the selected time point.

All Time Point is an option available only with time resolved datasets.

In the rebuild process the following structure of the filament creation is applied:

1. According to the setting of the **Keep Data** option the existing Filament structure is discarded or re-used
2. The selected Rebuild algorithm is completed
3. In the case of the time resolved data sets, according to the setting of **All Time Points** options, the parameters are either used to create the filament structure for the selected time point only or to create the filament structure for all time points.

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Button **Rebuild Objects**
Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Filament computation can be repeated for the same region, a different region, or the entire image. The Creation Parameters values are listed as a table.

In step three of the Creation wizard – Dendrite Point Diameter, a new option exists for selecting a **Starting Point**.

Use existing filament
Detect a new starting point

Use existing filament- the previously determined starting point will be used in the new filament structure. Therefore, in the next creation step (Starting point threshold) an option for Manual Start point editing in (shift+right click) will not be available.

Detect a new starting point -a new starting point of the filament will be determined, based on the value given for the largest diameter.

Remember Parameters Button

The Remember Parameters button allows you to save the complete set of the creation parameters and the relevant settings shown in the list.
Storing the parameters allows them to be easily reused, modified, or exchanged.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

Click on the Remember Parameters Button opens the Remember Creation Parameter window.

Remember Creation Parameter

Enter a name for the Creation Parameter set. This name identifies the Creation Parameter item in the Creation Parameter list used in the Edit/Preference/Creation Parameters and would be used as default name in the subsequent creation wizard algorithms.

14.5.6 Tab Draw

Method

When it is not possible to use Imaris automatic filament creation algorithms, either semi-automatic or manual creation will allow you to create filament objects by manually marking the object location in using method AutoPath, AutoDepth or Manual.

AutoPath
The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available. If you select this method please refer to chapter AutoPath (Surpass View - Filament - Draw - AutoPath).

AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed). If you select this method please refer to chapter AutoDepth (Surpass View - Filament - Draw - AutoDepth).

Manual
The manual segmentation feature is used to edit automatically traced Filaments or to build the Filaments manually from the image template. If you select this method please refer to chapter Manual (Surpass View - Filament - Draw - Manual).

Diameter

Here you adjust the filament diameter.

Type

Dendrite
Spine
Select as draw type Dendrite or Spine.

Button **Set Selection as Starting Point(s)**
If you select as Method **AutoPath** (see above, selection of the drawing Method) the button **Set Selection as Starting Point(s)** is available.

Select a point and click on this button to define the starting point of the filament.

Slice

If you select as Method **Manual** (see above, selection of the drawing Method) the Slice parameter settings are available.

YZ Plane
XZ Plane
XY Plane
Select the plane orientation of the drawing board: yz, xz, xy.

Automatic Placement
When this parameter is activated, the position is set automatically to the location of the highest data intensity. In order to avoid unwanted horizontal or vertical drawing offsets, the camera is set to a perpendicular position with an orthogonal projection. In this case the camera can only zoom or pan but not rotate.

x ... um
Display of the Slice the position.

Slider
The position of the drawing board can be altered in perpendicular direction by the Slice slider. The same is achieved using the Arrow-keys of the keyboard (Arrow up, Arrow down).

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

See also:
Menu Edit - Show Display Adjustment
Surpass View - Overview - Camera Toolbar - Pointer
Surpass View - Filament - Draw - AutoPath
Surpass View - Filament - Draw - AutoDepth

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14.5.6.1 AutoPath

The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available.

Drawing in AutoPath Mode on a PC:

- Select AutoPath and Shift + right-click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.
- Place the mouse cursor at the ends of the structure. The computed path is displayed.
- Shift + click and the displayed path is definitely drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

PC Mouse & Keyboard Function Overview in the AutoPath Mode

Shift + right-click	Set a starting point into volume
Set starting point, then move mouse	Display the path to the end point
Shift + left-click	The displayed path is definitely drawn
Mouse wheel	Change the "pencil" diameter for drawing the filament

Drawing in AutoPath Mode on a Mac:

- Select AutoPath and Shift + Ctrl + click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.
- Place the mouse cursor at the ends of the structure. The computed path is displayed.
- Shift + click and the displayed path is definitely drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

Mac Mouse & Keyboard Function Overview in the AutoPath Mode

Shift + Ctrl + click	Set a starting point into volume
Set starting point, then move mouse	Display the path to the end point
Shift + click	The displayed path is definitely drawn
Mouse wheel	Change the "pencil" diameter for drawing the filament

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.5.6.2 AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed).

Drawing in AutoDepth Mode on a PC:

- Select AutoDepth, press the Shift-key and hold down the left mouse button to draw a Filament. The depth is

automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. A pre-filtering would be then necessary.

PC Mouse & Keyboard Function Overview in the AutoDepth Mode

Shift + left-click & drag	Draw a filament into the volume
Mouse wheel	Change the "pencil" diameter for drawing the filament

Drawing in AutoDepth Mode on a Mac:

- Select AutoDepth, press the Shift-key and hold down the mouse button to draw a Filament. The depth is automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. A pre-filtering would be then necessary.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

Mac Mouse & Keyboard Function Overview in the AutoDepth Mode

Shift + click & drag	Draw a filament into the volume
Mouse wheel	Change the "pencil" diameter for drawing the filament

14.5.7 Tab Edit

Up to this point in the analysis process, most steps have been carried out automatically. You have influenced the tracing process by specifying a set of parameters. The Tab Edit allows you to make changes to the graph topology and the number of nodes and segments in the filament graph. You make these changes directly by processing part or all of the existing filament graph, or by operating on the 3D view of the graph.

Mouse Selects

Selecting a tool will change how the mouse interacts with the objects within the viewing area to match the properties of that selection. Specify how to select parts of the Filament by a single mouse click on the Filament graph.

Please Note: The selection of the filament type can be very different depending on which selection is chosen in the Track Edit tab. The selection is governed by the selection mode under both the Edit and Track Edit tab. The basis for the selection is the Mouse Selection mode found under in the Edit tab.

Expand selection on full Spines
Expand the initial selection set outwards from the original selected filament element until all attached spines objects are included in the selection.

Point
Point selects the smallest part of the segmented filament graph, only the position nearest to the mouse pointer.

Segment
Segment selects the graph section between two key vertices (branch points, beginning point, or ending points) of the same class, ether dendrites or spines.

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- Between the dendrite beginning point and dendrite branch point
- Between the dendrite branch points
- Between the dendrite branch point and dendrite terminal point
- Between the spine attachment point and spine terminal point
- Between the spine attachment point and spine branch point
- Between the spine branch point and spine terminal point

Branch
Branch selects the graph starting from the selected point to all the connected terminal points of the same class (a beginning point should be defined first for this mode).
Between the Dendrite Attachment or Dendrite Branch point to the Dendrite Terminal point for Dendrites.
Between the Spine Attachment point or Spine Branching point to the Spine Terminal point for Spines.

Filament
The Filament option selects the entire Filament graph.

Select Parts from Filament

There are various methods to make automatic selections on the whole Filament graph.

Button **Rel. Branch...**
Rel. Branch selects short parts of the Filament defined by the ratio of the branch length to trunk radius B/R (see figure above).

Button **Loops...**
Loops finds circular closed Filaments limited by a maximum length.

Button **Invert**
Invert selects all unselected parts of the graph and de-selects all selected parts.

Button **Abs. Branch**
Abs Branch selects all branches shorter than an absolute length B.

Button **Path**
Path requires at least two selected points from the Filament graph. The shortest path between these two points will be selected.

Button **Clear**
Clear removes the selection.

Process Selection

The following buttons apply only to the previously selected parts (see above) of the Filament.

Button **Assign as Dendrite**
Click on this button to assign the selection as dendrite.

Button **Assign as Spine**
Click on this button to assign the selection as spine.

Button **Assign as Dendrite Beginning Point**
Click on this button to assign the selection as dendrite beginning point.

Button **Smooth**
Removes the roughness of the current selection. It affects the position and the diameter of all segments except the branch points and end points.

Button **Center**
Click on this button to re-center the filament. The window **Center Selection** opens.
The position of the center axis is refined according to the peak intensity in the vicinity of the initial position.

Window:Center Selection

Source Channel
Select the respective source channel, from which the structure is to be created.

Button **Diameter...**
Click on this button to recalculate the filament diameter. The window **Calculate Diameter** (see below) opens.

Button **Duplicate**
Click on this button to copy the selection into a new Filament item. The new Filament item appears in the Surpass Tree.

Button **Join**
Click on this button to join two unconnected filament points or segments. The window **Center Join Selection on Channel** opens.
Select the objects to be joined in the image by Ctrl + left-click (Mac: Command+ left-click). The selected point or segment will be highlighted, then click Join. A new filament structure will be created structurally joining previously unconnected points or segments.

Window:Center Join Selection on Channel

Source Channel
Select the respective source channel, from which the Filament structure is to be created.

Button **Delete**
Click on this button to delete the current selection.

Window: Calculate Diameter

Source Channel
Select the respective source channel, from which the Filament structure is to be created.

Small Diameter
Type-in the smallest Filament diameter in the selected region.

Large Diameter
Type-in the largest Filament diameter in the selected region.

Contrast Threshold
Type-in the contrast threshold in the selected region.

Process Filament

The following buttons apply to the entire Filament.

Button **Smooth**
Smooth removes roughness from the Filament path. It affects the position and the diameter of all segments except the branch points and end points.

Button **Center**
Click on this button to re-center the filament. The position of the center axis is refined according to the peak intensity in the vicinity of the initial position.

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Button **Diameter...**
Click on this button to recalculate the Filament diameter. The window Calculate Diameter (see above) opens.

Button **Split**
Unconnected parts of the Filament can be split into separate Filament objects. The new objects will appear as new Items in the Surpass Tree. This allows individual coloring and individual statistics.

Button **Merge...**
In Imaris it its possible to merge two or several Filament objects into one Filament objects. This button is only available if you have two or more Filament items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Filament X" displays (see below).

Button **Export**
The whole Filament graph can be stored in the Neuron file format (*.hoc). The Neuron application is free and is able to do electrical simulations.

Window: Merge selected Objects to "Filament X"

Check the boxes in front of the desired Filaments or use the button **Select All**.

Button **Clear All**
Use this button to clear the Filaments selection.

Button **OK**
If you want to merge the Filaments click on **OK**.

Button **Cancel**
If you do not want to merge the Filaments click on **Cancel**.

Connect & Translate selected Edges (Vertices)
Check this box to connect and translate selected edges.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.5.7.1 Manual

The manual segmentation feature is used to edit automatically traced Filaments or to build the Filaments manually from the image template. Essentially you draw the Filaments onto the image, which serves as a visual reference (template). Drawing is done in the xy-plane. For z-positioning in the image you can choose to change planes manually (Arrow-keys or mouse) or to have the system change the planes automatically in such a way that the center of the Filament is kept in focus.

An orthogonal section plane appears within the Surpass viewer. Use the channel visibility and the Display Adjustments to optimize the section viewing. In order to draw Filaments onto the drawing plane switch the mode for the mouse pointer from Navigate to Select.

If you select Manual the Slice parameter settings are available.

Drawing in Manual Mode on a PC:

- Select Manual in the Draw box and press the Shift-key and hold down the left mouse button to begin drawing. The circle on the drawing plane indicates the Filament diameter.

The diameter can also be changed using the mouse wheel. To make a connection to an existing Filament, start painting on this Filament. If the filamentous structure runs perpendicular to the drawing plane, use the right mouse button to alter the plane orientation.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

PC Mouse & Keyboard Function Overview in the Manual Mode

Left-click plane & drag	Change plane position perpendicular to its orientation
Shift + left-click	Set a filament vertex
Shift + left-click plane & drag	Draw a filament
Right-click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the "pencil" diameter for drawing the filament
PageUp	Increase the "pencil" diameter for drawing the filament
PageDown	Decrease the "pencil" diameter for drawing the filament
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
Arrow up	Change slice position up
Arrow down	Change slice position down
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

Drawing in Manual Mode on a Mac:

- Select Manual in the Draw box and press the Shift-key and hold down the mouse button to begin drawing. The circle on the drawing plane indicates the Filament diameter.

The diameter can also be changed using the mouse wheel. To make a connection to an existing Filament, start painting on this Filament. If the filamentous structure runs perpendicular to the drawing plane, use the right mouse button to alter the plane orientation.

Mac Mouse & Keyboard Function Overview in the Manual Mode

Click plane & drag	Change plane position perpendicular to its orientation
Shift + click	Set a filament vertex
Shift + click plane & drag	Draw a filament
Ctrl + click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the "pencil" diameter for drawing the filament
PageUp	Increase the "pencil" diameter for drawing the filament
PageDown	Decrease the "pencil" diameter for drawing the filament
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

14.5.8 Tab Edit Tracks

Mouse Selects

This option allows you to select an object within an image according to its object type by a single mouse click. To edit the properties of multiple objects simultaneously, select the required object type.

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There are two options:
Filament
Track

Please Note: The selection of the filament type can be very different depending on which selection is chosen in the Track Edit tab. Selection is governed by the selection mode under both the Edit and Track Edit tab.

How to use the Selection Tools
Selection of different filament elements and tacks can be made within the Edit and **Track Edit** Tabs.

To make a suitable selection for time resolved filament images, you may need to make the object selection by clicking on the Mouse Selection options both under the **Edit** and **Tack Edit** tabs.
Once you have selected the Filament graph element under the **Edit** tab you can also use the selection options under the Track Edit tab to decide whether the object to be selected is just for **a single time point** or for **all time points**.

Under the Track Edit tab you can decide either to select objects for **a single time point** by choosing the option **Filament** or for **all time points** along the selected track by selecting the option **Track**.

For example, if under the Edit tab the mouse pointer is the Segment option and under the Edit Track tab the mouse selection is the Filament option, then the Segment will be selected for a single time point.
However, if you alter the selection under the Track Edit tab to the Track option, the particular Segment will be selected for all time points along that track.

Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.
The first row of the track-editing table displays the timeline. Each number represents a time point in the series.
The second row of the track-editing table displays the **object name**.
Multiple object types can be tracked easily since every object type appears in different colors.

Object ID

If the mouse pointer is on the object, a tooltip appears that identifies the object ID. The ID numbers of the objects are sequentially ordered column-wise from the top to the bottom of the table. The object maintains its unique ID number independent of the track being allocated to.
If the mouse pointer is on the connection, a tooltip appears that identifies the track ID number.

Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.
In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
For multiple selections press Ctrl+click (Mac: Command+click).

Visualization options

Zoom
Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.
Using the mouse wheel will have the same affect.
Pan
To move the track editing table click and hold the right mouse button while dragging the mouse. Release the

right mouse button to place the table on the chosen location.

Time indicator
The first row of the track-editing table displays the timeline. Each number represents a time point in the series. The current time point is indicated by vertical line highlighted with in current selection color.

To modify the time point, do one of the following:
1. Moving the slider in the Time bar modifies its position in time and changes the time point in the track editor.
2. Clicking on the time point within the track-editing table automatically moves the time line indicator to the corresponding point.
Double clicking on the time indicator, fixes the position of the current time point at the center of the track-editing table, regardless of the selected zoom or pan options)

Editing

Track Editor has several features that help you modify your tracks

Add tracks

To set the starting point of a new connection first you have to select the object.
Shift+left-click followed by a mouse movement on the object creates the new connection.
To establish a connection, drag the edge to the target object. If valid, the target object is highlighted (red).
Release the mouse button and the line segment clips automatically onto the new object, creating a new connection between the two objects.

Please note: The new connection is only possible between the objects of the same type. If you release the mouse button without being on a target object, the edge will disappear and a new connection will not be established.

A connection between objects of the same time point is also invalid.

Delete Tracks Edges

Tracks
Select the track and press backspace to delete the whole track.

Connections
To delete the connection, select the connection and press shift-click. The selected connection is deleted.

The process of deleting the connection splits the track. The first track part remains with the same ID number and stops at the time point of the deletion. The second part consists of the remaining deleted objects and the connections between them. It is automatically shifted to the bottom of the track-editing table and assigned a new track ID number.

Connect unconnected components

Manually added, or previously untracked objects are displayed as separated circles. The procedure of creating new connections and connecting the objects is unchanged.

Show selection (build working set)

This option allows you to work on subset of tracks.

Connect Track Button

Click this button to connect two existing tracks. Select two Spots in two different tracks at the subsequent time points (just one Spot per time point) and click on the button Connect Track. The connection is automatically created between all selected objects.

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Disconnect Track Button

Select a object and click on the button Disconnect Track. The connection from the selected object to the next object in time sequence is deleted.

Correct Drift

If you have an object drift (e.g. a cell drift) during the image acquisition you can use the Correct Drift button to correct the drift for the whole data set.

The ImarisTrack tracks all objects in an image, even the objects that should be stationary.
The Correct Drift function gives the user the opportunity:

1) to specify the tracks related to the stationary objects
2) to assign these objects as stationary objects and
3) to adjust the position of the rest of the image objects based on this selection.

As a result of drift correction, the entire image is transformed in such a way that the selected objects remain stationary and within the new image each objects' position (changes) based on the correct draft algorithm. Within the new image, the true movement of the objects can be then detected relative to the stationary objects.

In the accordance with the selected for **Result DataSets** options. the new image coordinates are calculated and displayed.

To make the Drift Correction on the data set the following steps are completed:

- Select the tracks associated with the stationary objects
- Select the algorithm for drift correction
- Select the option for the Result data sets
- Verify the selection and click on the OK button.

Please note: The drift correction changes the data set.

Based on the provided the track sub-selection the algorithm determines the new objects position for all time points.
If the tracks are selected, the algorithm will be performed on the sub selection. Otherwise, every track of the image is considered for calculations.

Algorithms

The Drift Correction is calculated based on the two algorithms, Translation and translation and rotation.

Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

Please note: The number of the selected tracks affects the algorithm calculation.

Selection of a single track gives a complete description of a translation.
Selection of a two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).
A better fit between the time points is achieved when three or more tracks are selected.

Result DataSet size

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.
Select the Result data set option to determine which new image part will be displayed.

Crop largest common region

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

New size equal to current size

The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

Include entire result

The final image will be the size of the combined area of the original and corrected image.
Selecting the appropriate Result dataset size may speed up the data visualization. This is especially important with (with large data sets.)

Correct spots positions

Activate this parameter and the spots are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.5.9 Tab Filter

Please note: Before using you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be

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used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Dendrite) is displayed at the bottom of the threshold histogram.

Duplicate Selection to New Filament Button
Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

See also:
Surpass View - Overview - Surpass Tree

14.5.10 Tab Statistics

Filament statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Filament. The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.
Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:
a) Overall
b) Detailed
c) Selection
In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.
To sort the columns click on the arrow in column heading. Click again to reverse the sort.

Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Detailed Tab

A pull down list offers three types of **Detailed** statistical calculations:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates

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a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values
The Average table contains values determined on the basis of all values within a set of the selected variable.
Columns are labeled as follows:
Min (Minimum)- the smallest value within a set of values of the selected variable
Max (Maximum)- the largest value within a set of values of the selected variable
Mean – value of the sum of all values divided by number of values within a set
Std Dev (Standard deviation)- a measure of the values distribution around the mean values
Median- the middle value of the sorted set of variables
Sum- the sum of all values within a variable set
Count- number of values within a set
Unit- variable unit
Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values
If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.

Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image elementthat has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).
A pull down list offers three types of statistical calculations from a Selection:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values
The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values
If the option All values is selected, all statistical values will be displayed for the selected image element.

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.
Click on the button **Settings** and you switch to the window Preferences - Statistics - Filament.

Button **Search** - Search by object ID.
Type in the object ID in the data field. Click on the button **Search**.
- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Filament** - Duplicate Selection to new Filament.
Click on this button and all selected elements are copied to a new Filament object. The new Filament appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation**
Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** or **All Values** are selected

Button **Export** - Export Statistics on Tab Display to File.
Only the statistics that are shown within the current tab will be exported.

Button **Export** - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.
All of the statistics (that are enabled in the statistics settings) will be exported.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:

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Menu Edit - Preferences... - Statistics - Filament

14.5.11 Tab Color

Tab Color edit, manage and assign colors material to the Filament object.

Object Type

Choice of Object type is presented in drop down window. Select either Dendrite or Spine and depending on the chosen object type selection of the variables is listed.

The Two color types are available:
Base
Statistics Coded

14.5.11.1 Color Type-Base

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.5.11.2 Color Type-Statistics Coded

Statistics Coded

Depending on the object type selection the Statistical variable selection is listed in the drop down window. For example, if Dendrite is chosen as the Object type, available Statistical type will be the Dendrite linked variable.

Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area.

Show Colorbar
Check the box to see the statistics colorbar in the viewing area.

Show Title
Check this box to see the title in the statistics colorbar.

Show Range
Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**
Click the **Reset** button to set the image back to the original values.

Button **Load** ...
Click on the button **Load** to display the Import Color Table File color window. Enter the path and the requested File name and/or File type and click Open. The selected file is loaded and displayed.

Colormap range

Colormap range-specify the limits for colorbar.
Min - lower color limit
Max - upper color limit

Button **Auto**
Click the button **Auto** to detect the system the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

14.5.12 Mouse & Keyboard PC

Filament Mouse & Keyboard Functions on the Tab Draw

AutoPath Mode

Shift + right-click	Set a starting point into volume
Set starting point, then move mouse	Display the path to the end point
Shift + left-click	The displayed path is definitely drawn
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

AutoDepth Mode

Shift + left-click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

Manual Mode

Left-click plane & drag	Change plane position perpendicular to its orientation
Shift + left-click	Set a filament vertex
Shift + left-click plane & drag	Draw a filament
Right-click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

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PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
Arrow up	Change slice position up
Arrow down	Change slice position down
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:
Addendum - Mouse & Keyboard PC

14.5.13 Mouse & Keyboard Mac

Filament Mouse & Keyboard Functions on the Tab Draw

AutoPath Mode

Shift + Ctrl + click	Set a starting point into volume
Set starting point, then move mouse	Display the path to the end point
Shift + click	The displayed path is definitely drawn
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

AutoDepth Mode

Shift + click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

Manual Mode

Click plane & drag	Change plane position perpendicular to its orientation
Shift + click	Set a filament vertex
Shift + click plane & drag	Draw a filament
Ctrl + click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)
PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:
Addendum - Mouse & Keyboard Mac

14.6 Frame

The Frame serves to outline the object in the viewing area. In the Surpass Tree check the check-box Frame to display the frame in the viewing area. Uncheck the box to make the frame invisible.

How to Add a new Frame?

- Select menu Surpass - Frame.

The Surpass Tree list is automatically updated and in the Frame - Properties the Settings parameter are displayed.

14.6.1 Tab Settings

Highlight Frame in the Surpass Tree and click on the tab Settings. The Frame Settings display in the properties area.

Box and Time Settings

Box
Check the parameter to display a box around the Frame.

Line Width
Adjust the box line width with the respective slider.Moving the slider to the left reduces the line width, moving it to the right increase line width.

Shading
Check this parameter to display a shading. Use the slider to adjust the shade contrast.

Shadow
The entire Surpass scene can produce shadows on the three planes of the Frame object. Check this box to display the shadows. Use the Shading slider (see above) to adjust the contrast. The rendering is done in real-time, and it requires a good performance of the graphics board (depending on the complexity of the scene).

Time Colorbar
Only available if you work with a time data set. Check this box and the Time Colorbar displays in the lower left corner of the viewing area.

Grid and Tickmarks Settings

Grid
Check the box Grid to display a grid. Use the slider to adjust the grid width.
Scale is shown using a grid behind the object. The frame moves to stay behind the object as it is rotated. The distance represented by each square in the grid, the scale bar, is shown in the bottom right corner of the screen.

Tickmarks
Check the box Tickmarks to display tickmarks on the edges of the frame.

Fix to Data Set
Check Fix to Data Set to fix the Frame to the same side when navigating the object.

Spacing
Specify the grid and tickmark spacing.

Labels Settings

Axis Labels
Check the box Axis Labels to display the x, y, and z axes to clearly show the orientation of the object. If this box is checked the parameter Label Color and Label Font is available (see below).

Label Color
Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Color Editor

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window to change the color of the label on the coordinate axes.

Label Font
Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Font Editor window to change the font to label the coordinate axes.

14.6.2 Tab Color

Tab Color edit, manage and assign colors material to the Frame.

14.7 Group

Groups are folders in the Surpass Tree that contain other Surpass objects. They are visible in the objects area and allow you to create a treelike hierarchy of Surpass objects. When the first Surpass object is created, it is automatically placed in a Group called Surpass Scene. Additional Groups can be created in order to sort objects of the scene.

How to Add a new Group?

- Select menu Surpass - Group.

The Surpass Tree list is automatically updated and in the Group - Properties the Settings parameter are displayed.

How to Add Items in a new Group?

- Click on an Item in the Surpass Tree list and drag it onto the Group object. Once released, the Item is added to the Group.

Like all other objects, Groups have a visibility check-box. This box affects the visibility of all objects in the Group.

See also:
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Scene File Concept

14.7.1 Tab Settings

Number of Objects
Displays the number of objects in the Group.

Button **Show All**
If you click this button the check-box(es) of the Item(s) in the Group are checked.

Button **Hide All**
If you click this button the check-box(es) of the Item(s) in the Group are unchecked.

14.7.2 Tab Color- Base

Color Tab edit, manage and assign material colors of an Object.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.7.3 Tab Statistics

The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations. To perform further analysis of large amounts of statistical data, use the Group Level Statistic. The Statistics tab collects and displays the Group's statistical values, which are lists of the statistical value summaries of all created objects contained in the specified Group.
For further analysis select the Export button and the statistic values of all objects are exported as a single file.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:
a) Overall
b) Detailed

In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Detailed Tab

A pull down list offers three types of Detailed statistical calculations:
Specific Values
Average Values
All Values

Time Plots

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The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values
The Average table contains values determined on the basis of all values within a set of the selected variable.
Columns are labeled as follows:
Min (Minimum)- the smallest value within a set of values of the selected variable
Max (Maximum)- the largest value within a set of values of the selected variable
Mean – value of the sum of all values divided by number of values within a set
Std Dev (Standard deviation)- a measure of the values distribution around the mean values
Median- the middle value of the sorted set of variables
Sum- the sum of all values within a variable set
Count- number of values within a set
Unit- variable unit
Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Settings Button -Configure a List of visible statistics values
Click on the button Settings and switch to the window Preferences

Search Button - Search by object ID
Type in the object ID in the data field. Click on the button Search.
Highlights the object in the viewing area
Highlights the object on the Detailed Tab
Displays of the values on the Selection Tab

Export Button - Export Statistics on Tab Display to File
Only the statistics that are shown within the current tab will be exported.

Button **Export** - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Export Button - Export All Statistics to File
All of the statistics (that are enabled in the statistics settings) will be exported.
With a click on the button Export the window Export Statistics opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

14.8 Light Source

A Light Source serves to illuminate objects in the viewing area. A light source node serves as the location and orientation of a light source.

How to Add a new Light Source?

- Select menu Surpass - Light Source.

The Surpass Tree list is automatically updated and in the Light Source - Properties the Settings parameter are displayed. Multiple light sources can be added in the Surpass Tree.

Move Light Source

To change the Light Source position and direction, choose the pointer mode Select and drag the cone or line.

Light Intensity

Click on the cylinder above the cone and move it closer or further away from the object to change the light intensity.

See also:
Surpass View - Overview - Surpass Tree
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)

14.8.1 Tab Settings

Fix to Data Set
Check the box to fix the Light Source to the same side when navigating the object. When the box is unchecked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

14.8.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.8.3 Mouse & Keyboard PC

Light Source Mouse & Keyboard Functions

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Left-click cone (or line) & drag	Change direction
Left-click cylinder & drag	Change intensity

See also:
Addendum - Mouse & Keyboard PC

14.8.4 Mouse & Keyboard Mac

Light Source Mouse & Keyboard Functions

Click cone (or line) & drag	Change direction
Click cylinder & drag	Change intensity

See also:
Addendum - Mouse & Keyboard Mac

14.9 Measurement Point

How to Add a new Measurement Point?

- Select menu Surpass - Measurement Point.

The Surpass Tree list is automatically updated and in the Measurement Point - Properties the Edit parameters are displayed.

14.9.1 Tab Settings

Point Shape

Tetrahedron
Cube
Sphere
Here you can choose from three different shapes for the Measurement Points (tetrahedron, cube, or sphere).

Filled
Check the box and the point shape is solid.

Point Diameter

Here you can define the Measurement Point diameter.

Point Labels

Name
Check the check-box to display the name of a Measurement Point in the viewing area.

Intensity
Check the check-box to display the intensity of a Measurement Point in the viewing area. The intensity of the channel is displayed in brackets.

Line Mode

Pairs (AB, CD, ...)
Any two consecutive points are connected by a line.

Polygon (ABCD ...)
All points in the Group are connected consecutively (P1-P2-P3-P4, etc.).

Line Width

Here you define the line width.

Line Labels

Distance
The measured distance between two points is displayed next to the line connecting the two Measurement Points. Check the check-box to display the distances in the viewing area.

Velocity
Only accessible if you have a time data set. Check the box to display the speediness between two Measurement Points.

Tip: If you do not have the module ImarisTrack you can easily use the velocity function to track single objects over several time points and measure the velocity.

Button Color
Button Font
Label Color and Font
To change the color or font of the label (display of the line length) click on the respective icon.

Tip: You can find the parameters to change the color of the Measurement Points and connecting lines on the tab Colors.

Visible for all Time Points
Only accessible if you have a time data set. Check the box to display the inserted Measurement Points, lines, and annotations at all time points.

See also:
Surpass View - Measurement Point - Tab Color

14.9.2 Tab Edit

Autodepth Position

In the mouse pointer mode Select:
Shift-click with the left mouse button to add a new point. Left-click to select an existing point. Shift-left-click to reposition a selected point.

Intersect with

The first two parameters are relevant if you want to insert Measurement Points in a volume rendered data set. For the rest the last parameter is crucial.

all visible Channel
In the Display Adjustments you decide, whether a channel is visible or not in the viewing area. Select this parameter and all visible channel are relevant for the placement of the Measurement Points.

specific Channels
In the Display Adjustments you decide, whether a channel is visible or not in the viewing area. Select this option and choose the channel directly from the drop down list.
The selected channel is relevant for the placement of the Measurement Points.

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solid Objects
Check this box and the Measurement Points are placed on the surface of an object. Uncheck the box and you can also place Measurement Points in an object.

Button **Delete All Points**
Click on this button to delete all Measurement Points. Alternatively click on the Delete-key on the keyboard.

Selected Point

Click to select an existing point. If no point is selected the following parameters are grayed. Re-click on a selected point to un-select the point.

Button **Delete**
Click on this button to delete the selected Measurement Point.

Name
Text field to edit the Measurement Point name.

Position
X
Y
Z
T
Data field of the x-, y-, z- and t-coordinates.

See also:
Menu Edit - Show Display Adjustment
Surpass View - Overview - Camera Toolbar (Pointer Select)

14.9.3 Tab Intensity

Imaris reads out the intensity profile of each voxel against the distance. Here you select the channels that should be displayed in the histogram and you choose the desired connecting lines between the Measurement Points.

Histogram

The histogram displays the intensity profile along the lines. The labeled lines represent the Measurement Points. The x-axis represents the length of the profile. On the left hand side of the histogram you find the display of the channel(s) intensity range. The channel selection is located below the histogram. Here you can switch channels in the intensity profile on or off. Below the channel selection you find the profile selection. In the drop-down list select the desired line and the histogram updates accordingly.

Button **Snapshot**
Click on this button to do a Snapshot of the histogram. The window Save Snapshot as... opens.

See also:
Menu File - Save as... Snapshot

14.9.4 Tab Statistics

Measurement Point statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Measurement Points. The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

a) Overall
b) Detailed
c) Selection

In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.
To sort the columns click on the arrow in column heading. Click again to reverse the sort.

Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Detailed Tab

A pull down list offers three types of **Detailed** statistical calculations:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

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Average values
The Average table contains values determined on the basis of all values within a set of the selected variable.
Columns are labeled as follows:
Min (Minimum)- the smallest value within a set of values of the selected variable
Max (Maximum)- the largest value within a set of values of the selected variable
Mean – value of the sum of all values divided by number of values within a set
Std Dev (Standard deviation)- a measure of the values distribution around the mean values
Median- the middle value of the sorted set of variables
Sum- the sum of all values within a variable set
Count- number of values within a set
Unit- variable unit
Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values
If the option All values is selected for the detailed tab, all values will be displayed. Even thought this option is always readily available, it is discouraged to use it in order to avoid data clutter.

Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image elementthat has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).
A pull down list offers three types of statistical calculations from a Selection:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Average values
The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light**

gray area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.
Click on the button **Settings** and you switch to the window Preferences - Statistics - Measurement Points.

Button **Search** - Search by object ID.
Type in the object ID in the data field. Click on the button **Search**.
- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Measurement Points** - Duplicate Selection to new Measurement Points.
Click on this button and all selected elements are copied to a new object. The new Measurement Points appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Export** - Export Statistics on Tab Display to File.
Only the statistics that are shown within the current tab will be exported.

Button **Export** - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.
All of the statistics (that are enabled in the statistics settings) will be exported.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

Buttons Overview

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click

See also:
Menu Edit - Preferences... - Statistics - Measurement Points

14.9.5 Tab Color

Color Tab edit, manage and assign material colors of an Object.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

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Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.9.6 Mouse & Keyboard PC

Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + left-click	Add a new measurement point
Shift + left-click	Reposition selected measurement point
Left-click on existing point	Select measurement point
Mouse wheel in Add mode	Turn wheel up: Increase size of next measurement point
	Turn wheel down: Decrease size of next measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of the shape around the mouse pointer changes accordingly. This helps a lot in handling 3D data sets.

See also:
Menu Edit - Preferences ... - 3D Cursor (adjust 3D Cursor Shape)
Addendum - Mouse & Keyboard PC

14.9.7 Mouse & Keyboard Mac

Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + click	Add a new measurement point
Shift + click	Reposition selected measurement point
Click on existing point	Select measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of shape around the mouse pointer changes accordingly. This helps a lot in handling 3D data sets.

See also:
Menu Edit - Preferences ... - 3D Cursor (adjust 3D Cursor Shape)
Addendum - Mouse & Keyboard Mac

14.10 Oblique Slicer

The Oblique Slicer represents a plane that can be freely moved and rotated within the data set and allows you to look inside any object. The Oblique Slicer consists of a colored frame with a white double rod in the middle.

How to Add a new Ortho Slicer?

- Select menu Surpass - Oblique Slicer.

The Surpass Tree list is automatically updated and in the Oblique Slicer - Properties the Settings parameter are displayed. The Oblique Slicer is displayed in the viewing area (depending on the default parameter on the tab Settings).

Change Position of the Oblique Slicer

You can change the position of the Oblique Slicer. Choose the pointer mode Select, highlight the Oblique Slicer in the viewing area and click on the bigger diameter rod. It will turn colored as it is selected, move it to set the Oblique Slicer to the required position.

Rotate Oblique Slicer around Axes

The Oblique Slicer can be rotated around the x-, y-, and the z-axes. In Select mode click the smaller diameter rod. It will be highlighted as it is selected. To rotate the Oblique Slicer, move the smaller rod.

See also:
Surpass View - Overview - Camera Toolbar - Pointer (Mode Select)
Surpass View - Ortho Slicer - Tab Settings

14.10.1 Tab Settings

Orientation

Button **YZ Plane**
Button **XZ Plane**
Button **XY Plane**
Change the Slicer direction, if required, by selecting the adequate plane.

Position

Button **Recenter**
Click on this button to re-center the Slicer.

Extended Section

The section is a single cut through the data set, the highlighted lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

Show Frame
Check the box to display a colored frame around the Slicer.

Camera

Button **Orthogonal View**
The camera is set to a position which is perpendicular to the Slicer.

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14.10.2 Mouse & Keyboard PC

Oblique Slicer Mouse & Keyboard Functions

Left-click on the bigger diameter rod & drag	Move plane to desired position
Left-click on the smaller diameter rod & drag	Rotate plane to desired position

See also:
Addendum - Mouse & Keyboard PC

14.10.3 Mouse & Keyboard Mac

Oblique Slicer Mouse & Keyboard Functions

Click on the bigger diameter rod & drag	Move plane to desired position
Click on the smaller diameter rod & drag	Rotate plane to desired position

See also:
Addendum - Mouse & Keyboard Mac

14.11 Ortho Slicer

Ortho Slicer represent an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. When adding slices, Surpass suggests per default the directions z, x, and y as sequence for the first three and all subsequent slices.

How to Add a new Ortho Slicer?

- Select menu Surpass - Ortho Slicer.

The Surpass Tree list is automatically updated and in the Ortho Slicer - Properties the Settings parameter are displayed. The first Slice is displayed in the viewing area (depending on the default parameter on the tab Settings).

See also:
Surpass View - Ortho Slicer - Tab Settings

14.11.1 Tab Settings

Slice Orientation

YZ Plane
XZ Plane
XY Plane
The check XY, XZ and YZ plane, to select the visibility of the corresponding slice.

Extended Section

The section is a single cut through the data set, the colored lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

Show Frame
Check the box to display a colored frame around the active slice.

Slice Position

Slider
Choose the relative position of the Slice in the data set by means of the slider or a direct entry of the slice position given as the offset distance from the data set origin (equals the absolute coordinates).

14.11.2 Mouse & Keyboard PC

Ortho Slicer Mouse & Keyboard Functions

Left-click plane & drag	Change slice position
Right-click plane	Change slice orientation
Num plus	Increase the extended section
Num minus	Decrease the extended section
Arrow up	Change slice position up
Arrow down	Change slice position down

See also:
Addendum - Mouse & Keyboard PC

14.11.3 Mouse & Keyboard Mac

Ortho Slicer Mouse & Keyboard Functions

Click plane & drag	Change slice position
Ctrl + click plane	Change slice orientation
Num plus	Increase the extended section
Num minus	Decrease the extended section

See also:
Addendum - Mouse & Keyboard Mac

14.12 Spots

The Spots object is available to model point-like structures in the data. It provides a procedure to automatically detect point-like structures, an editor to manually correct detection errors, a viewer to visualize the point-like structures as spheres, and statistics output.

How to Add new Spots?

- Select menu Surpass - Spots.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Spots - Properties.

See also:
Suprass View - Spots - Creation Wizard

14.12.1 Creation Wizard

The Creation Wizard allows to go forth and back until the optimal parameters are found.
In the first window of the Spots Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.
The most convenient way to apply the same creation parameters to different data sets is to use the **Load Parameters** option.

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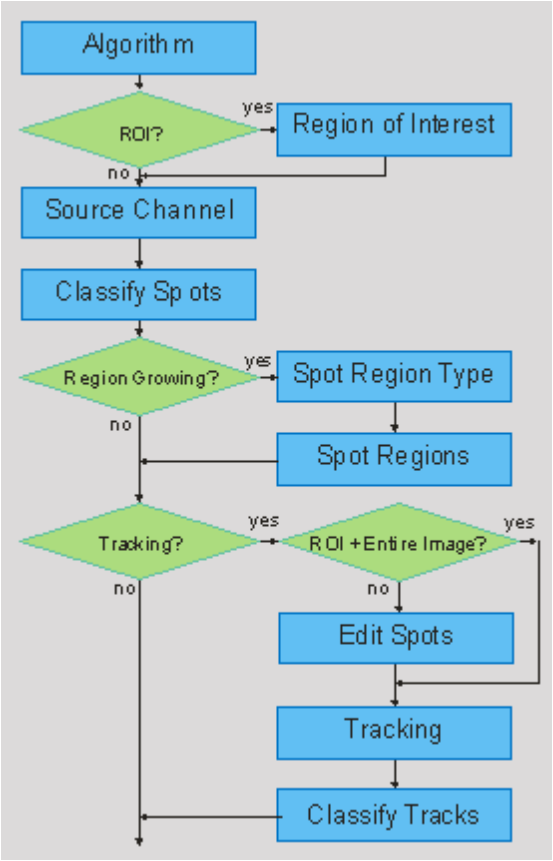
This is the flow diagram of the Spots Creation Wizard:



In the first window of the Creation Wizard you find the algorithm settings. Here you have to decide, if you want to segment only a **Region of Interest (ROI)**, if you want to use the **Region Growing** for the Spot detection, and if you want to create **Tracks** after the **Spot** detection.

For further details please refer to chapter Algorithm, Region of Interest, Source Channel, Classify Spots, Spot Region Type, Spot Regions, Edit Spots, Tracking, and Classify Tracks.

14.12.1.1 Algorithm

Algorithm Settings

If you do not want to use the automatic Spot creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button

Terminates the creation wizard and activates the manual Spots creation.

Load Parameters

This option lets you use the identical creation parameters for multiple datasets.
The option to Load Parameters is only available if the set of the creation parameters has already been saved under the Creation tab using the Remember Parameters button.
Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are

automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Load Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

If you do not want to use the automatic Cell creation wizard select the **Skip automatic creation, edit manually** button. If you do not want to use the automatic Cells creation wizard select the **Skip automatic creation, edit manually** button.

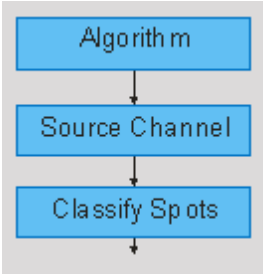
Segment only a Region of Interest
A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.
Check this box if you want to segment only a Region of Interest. If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally
Process entire Image finally this option appears only, if Segment only a Region of Interest is checked.
Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters (Complete ROI). Uncheck this box and only the region of interest is processed.

Different Spot Sizes (Region Growing)
Check this box if you want to apply the region growing, starting on the tab Spot Region Type.

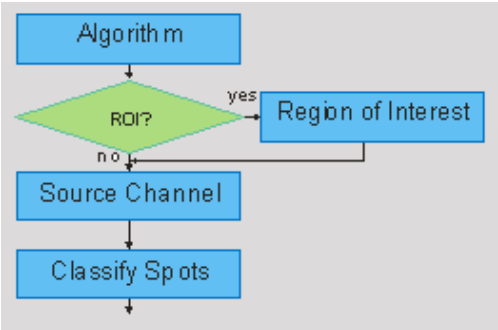
Track Spots (over Time)
Check this box if you want to track Spots over time, for details please refer to tab Tracking.

Some Examples



nothing checked

Structure of the Creation Wizard is:
- 1/3 Algorithm
- 2/3 Source Channel
- 3/3 Classify Spots



ROI

Structure of the Creation Wizard is:
- 1/4 Algorithm
- 2/4 Region of Interest
- 3/4 Source Channel
- 4/4 Classify Spots

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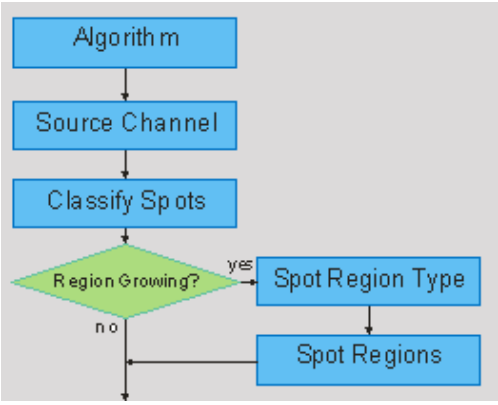
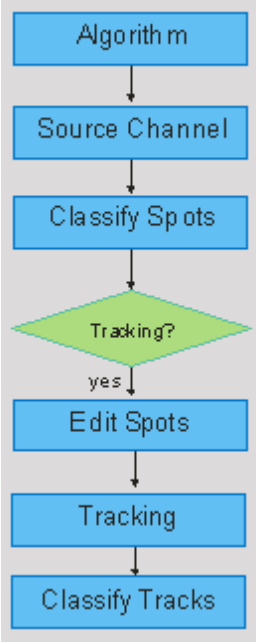


Region Growing

Structure of the Creation Wizard is:

- 1/5 Algorithm
- 2/5 Source Channel
- 3/5 Classify Spots
- 4/5 Spot Region Type
- 5/5 Spot Regions

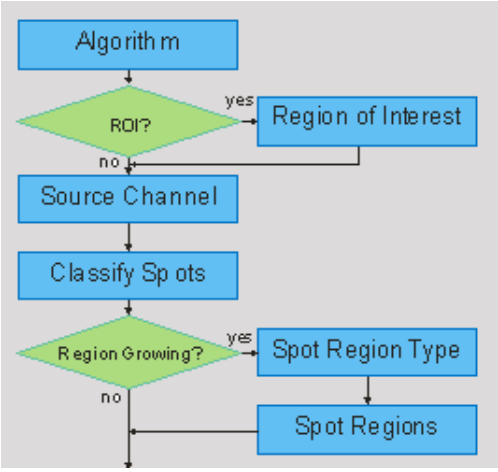


Tracking

Structure of the Creation Wizard is:

- 1/6 Algorithm
- 2/6 Source Channel
- 3/6 Classify Spots
- 4/6 Edit Spots
- 5/6 Tracking
- 6/6 Classify Tracks

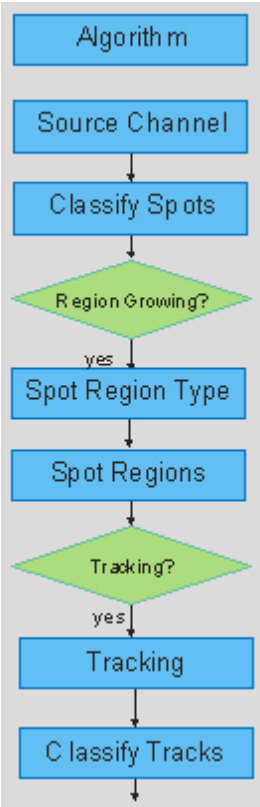
Combinations



ROI and Region Growing

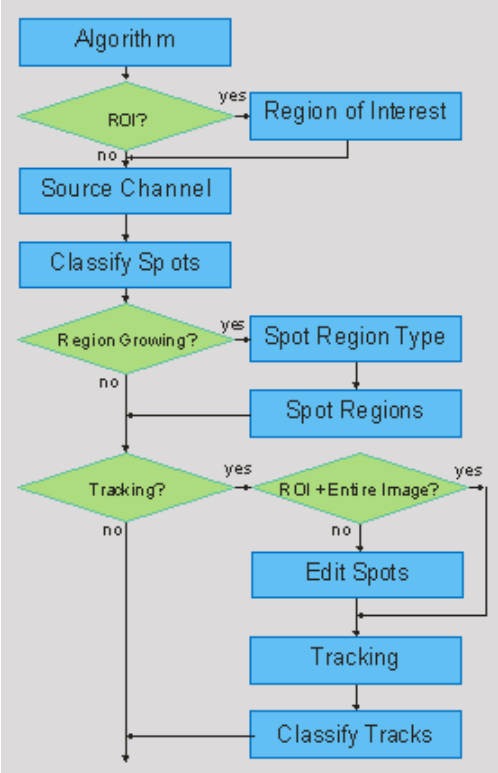
Structure of the Creation Wizard is:

- 1/6 Algorithm
- 2/6 Region of Interest
- 3/6 Source Channel
- 4/6 Classify Spots
- 5/6 Spot Region Type
- 6/6 Spot Regions



Growing and Tracking

Structure of the Creation Wizard is:
1/7 Algorithm
2/7 Source Channel
3/7 Classify Spots
4/7 Spot Region Type
5/7 Spot Regions
6/7 Tracking
7/7 Classify Tracks



- ROI, Region Growing and Tracking
- not Entire Image

Structure of the Creation Wizard is:
- 1/9 Algorithm
- 2/9 Region of Interest
- 3/9 Source Channel
- 4/9 Classify Spots
- 5/9 Spot Region Type
- 6/9 Spot Regions
- 7/9 Edit Spots
- 8/9 Tracking
- 9/9 Classify Tracks

- ROI, Region Growing and Tracking
- plus Entire Image

Structure of the Creation Wizard is:
- 1/9 Algorithm
- 2/9 Region of Interest
- 3/9 Source Channel
- 4/9 Classify Spots
- 5/9 Spot Region Type
- 6/9 Spot Regions
- 7/9 Tracking
- 8/9 Classify Tracks
- 9/9 Complete ROI

Spots Wizard Buttons

Button **Back**
This button is grayed in the first window of the Creation Wizard.

Button **Next**

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Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Spots click on **Cancel**.

14.12.1.2 Region of Interest

Region of Interest 1

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

X, Y, Z, Time
Min, Max, Size
Minimum, maximum and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields.
Alternatively the location and size of the ROI can be adjusted directly in the viewing area.
Size of the ROI:
1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X ,Y and Z.
Location of the ROI:
1.Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.
The numbers in the value fields are updated automatically.

Memory Usage:
Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button **Add**
Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button **Delete**
Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

Total Memory Usage:
Display of the memory usage for the image size within the ROI.

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Spots automatically click on **Cancel**.

See also:
Surpass View - Overview - Camera Toolbar (change to pointer mode Select)

14.12.1.3 Source Channel

Source Channel

Select the respective source channel, from which the Spots are to be computed.

Spot Detection

Estimated Diameter
Objects with size smaller than specified **Estimated Diameter** are not detected. This is also the diameter of the inserted Spots.

Background Object Subtraction
If checked, the background will be removed prior to spot detection. Technically background subtraction smoothes the image. The intensity is the one of a Gaussian filtered channel (Gaussian filtered by 3/4) minus the intensity of the original channel Gaussian filtered by 8/9 of sphere radius.

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Spots click on **Cancel**.

14.12.1.4 Classify Spots

The Spots filtering is incorporated into the creation process.

A filtering allows the removal of objects that would interfere with interpretation of results.

Quality: The 'Quality' is the intensity at the center of the spot in the channel the spot was detected.

If 'Background Subtraction' was not checked this is the intensity of the channel Gaussian filtered by 3/4 of spot radius.
If 'Background Subtraction' was active the intensity is the one of a Gaussian filtered channel from above minus the intensity of the original channel Gaussian filtered by 8/9 of spot radius.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

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In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Spots click on **Cancel**.

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14.12.1.5 Spot Region Type

This tab only appears, if **Different Spot Sizes (Region Growing)** is checked (Algorithm Settings).

The region method for segmentation in Imaris starts with defined seed points. You use Spots as seed points for the region growing that is performed in the next steps. During the region growing process around each seed point the region grows until a border stops the growing.

Spot Regions from

Absolute Intensity
Local Contrast
You can set the border using the Absolute Intensity or the Local Contrast as criteria. In both cases as next step the selected channel is displayed in the viewing area.

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Spots click on **Cancel**.

14.12.1.6 Spot Region

This tab only appears, if **Different Spot Sizes (Region Growing)** is checked (Algorithm Settings).

Spot Regions

The working channel is visualized in the viewing area, the histogram is displayed in the properties area. Regions above the threshold are displayed in white, regions below threshold are displayed in black. Depending on the previously selected detection method (Absolute Intensity or Local Contrast) refer to Absolute Intensity or Local Contrast.

Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Absolute Intensity

Automatic
In the working channel a copy of the intensity histogram of the source channel is displayed. Regions above the threshold are displayed in white. Now you adjust the threshold of the working channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

Local Contrast

Automatic
In the selected channel the regions with a local contrast brighter than the background are displayed in white. Now you adjust the threshold value of the local contrast channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

Diameter from

Region Volume
The Spot(s) radius is calculated from the region volume (the region volume and the Spot volume are equal).

Region Border
The radius of the calculated Spot(s) is the smallest distance from each Spot center to the region border.

Create Color Coded Region Channel
Additional to the Spots the Region Channel is displayed. Each region has a different color.

Preview

Here you select the preview settings.

Ortho Slicer
Check this box if you want to see the ortho slicer in the preview.

Volume Rendering
Check this box if you want to see a volume rendering in the preview.

Slicer Plane
XY, XZ, YZ
If you check the box Ortho Slicer (see above) these radio buttons are available to select the slicer plane.

Show Spots
Check this box to see the spots in the preview.

Button **Change Regions Color**
Click on this button to change the regions color.

Spots Wizard Buttons

Button **Back**

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With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Spots click on **Cancel**.

14.12.1.7 Edit Spots

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Autodepth Draw

Intersect with
The auto depth function will automatically calculate the Spot depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Spot.

all visible Channel
specific Channel - selected channel will be used for Spots depth computation
solid Objects
Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

Process Selection

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new Spot	Shift + left-click	Shift + click
Delete Spot	Left-click to select, then click on the the button Delete or Shift + left-click on a selected Spot	Click to select, then click on the the button Delete or Shift + click on a selected Spot
Change Spot diameter	Mouse wheel	Mouse wheel

Button **Duplicate**
Click this button and all selected Spots appear in a new group in the Surpass Tree (Spots Selection).

Button **Recenter**
Click on the Recenter button results in change of the selected Spots position. The new center is placed in position of the local intensity maximum in the vicinity of the initial spot center.

Button **Delete**
Click on this button to delete the selected Spot(s).

Button **Clear**
Click this button to clear the Spot selection.

No Spot Selected/Selected Spot

Position X: Y: Z: [um]
Display of the Spot position.

Diameter [um]
The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

Time Index
The number indicates the current time point in the series for the selected object.

Spots

In Imaris it its possible to merge two or several Spots items into one Spots item.

Button **Merge ...**
This button is only available if you have two or more Spots items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Spots X" displays (see below).

Window: Merge selected Objects to "Spots X"

Check the boxes in front of the desired Spots items or use the button **Select All**.

Button **Clear All**
Use this button to clear the Spots selection.

Button **OK**
If you want to merge the Spots click on **OK**.

Button **Cancel**
If you do not want to merge the Spots click on **Cancel**.

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Tracks click on **Cancel**.

See also:
Surpass View - Overview - Camera Toolbar (Pointer Navigate/Select)

14.12.1.8 Tracking

Tracking allows objects to be traced throughout sequential frames of a time datasets, so each object maintains a unique identity in the output measurements.
This tab only appears, if **Track Spots (over Time)** is checked (Algorithm Settings).

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Algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Spots. Based on these predicted positions, the best matches to candidate Spots can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while Autoregressive Expert mode combines changes in position and intensity to compute total cost.

Brownian Motion
This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernable pattern).
The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion
This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction. The user-specified maximum distance value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert
This algorithm models the motion of each Spot as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

Connected Components
For this algorithm, Spots in adjacent time-points are considered connected if the Spot spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. The object with the greatest amount of overlap will be assigned the same track ID.
This is the only algorithm that automatically handles lineage (spots that split or merge). It will only work with images that have excellent time resolution, because the Spot cannot ever jump a distance larger than its own size.

Parameters

Max Distance
The maximum search distance that a tracked object are looked for is specified with the Max Distance value.
This parameter disallows connections between a Spot and a candidate match if the distance between the predicted future position of the Spot and the candidate position exceeds the maximum distance.
The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size file.

The **Max Gap Size** defines the maximum number of the consecutive time points witch are allowed to be missing in order to a track to be continues.

The parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see above).

Fill the gaps with all detected objects

If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

Intensity Weight
Based on the information obtained from the measured Spots intensity and position, the Intensity Weight function is used to balance the relative influence of the Intensity and Distance costs. The Total Cost is determined by combining the Intensity term and Distance term, and minimization of the Total Cost is used to determine the best track from all feasible ones.

TotalCost = Σ(all connections) DistanceCost + IntensityWeight * IntensityCost

The parameter is only available if you select Autoregressive Motion Expert (see above).

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Tracks automatically click on **Cancel**.

14.12.1.9 Classify Tracks

The Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Spots (over Time)** is checked (Algorithm Settings).

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

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By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated. Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Spots Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

Button **Cancel**
If you do not want to create Tracks click on **Cancel**.

14.12.1.1 Complete ROI

This tab only appears, if **Process entire Image finally** is checked (Algorithm Settings).

Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Spots Wizard Buttons

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Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
This button is grayed in the last window of the Creation Wizard.

Button **Finish**
Click on **Finish** to end the calculation and display the results in the viewing area.

Button **Cancel**
If you do not want to create Spots/Tracks click on **Cancel**.

14.12.2 Tab Settings

On the tab Settings you can adjust the rendering quality to produce faster performance of the display when the number of Spots is very large. The size of the Spots can be changed via the Radius Scale box.

Spots Style/Quality

Off
Select this option to disable the visualization of the Spots.

Sphere
This option displays the Spots as as spheres.

Center Point
This option displays the Spots as center points.

Please note: The computation of Spheres is time consuming. If you have many Spots in your data set Imaris automatically switches to the display mode Center Points. If you would like to switch to the Spheres mode again select Sphere and confirm the changes.

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Off
Select this option to disable the visualization of the Tracks.

Line
This option displays the tracks connections dawn as lines.

Cylinder
This option displays the tracks connections dawn as cylinders.

Width
Diameter
Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality
When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Tracks Path

Color Editor
Select a desired color for the Track display.

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Radius Scale
Edit field for the displacement vector radius.

Color Editor
Select a desired color for the displacement arrow.

Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider
Adjust the number of time points using the slider.

14.12.3 Tab Creation

Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list on which step of the Spots Creation Wizard you want to re-enter the creation process.

For details please refer to chapter:
Menu Surpass - Spots - Creation Wizard - Algorithm
Menu Surpass - Spots - Creation Wizard - Region Growing
Menu Surpass - Spots - Creation Wizard - Tracking

Button **Rebuild Objects**
Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Spots computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

Remember Parameters Button

The Remember Parameters button allows you to save the complete set of the creation parameters and the relevant settings shown in the list.
Storing the parameters allows them to be easily reused, modified, or exchanged.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

Click on the Remember Parameters Button opens the Remember Creation Parameter window.

Remember Creation Parameter

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Enter a name for the Creation Parameter set. This name identifies the Creation Parameter item in the Creation Parameter list used in the Edit/Preference/Creation Parameters and would be used as default name in the subsequent creation wizard algorithms.

Add to Batch Button
Add to Batch button places the command into Batch coordinator with all adjusted parameter values.

14.12.4 Tab Edit

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Autodepth Draw

Intersect with
The auto depth function will automatically calculate the Spot depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Spot.

all visible Channel
specific Channel - selected channel will be used for Spots computation
solid Objects
Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

Selection

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new Spot	Shift + left-click	Shift + click
Delete Spot	Left-click to select, then click on the the button Delete or Shift + left-click on a selected Spot	Click to select, then click on the the button Delete or Shift + click on a selected Spot
Change Spot diameter	Mouse wheel	Mouse wheel

Duplicate Button
Click this button and all selected Spots appear in a new group in the Surpass Tree (Spots Selection).

Recenter Button
Clicking on the Recenter button changes the position of the selected Spots. The new center is placed in the position of the local intensity maximum, which is in the vicinity of the initial spot center.

Button Delete
Click on this button to delete the selected Spot(s).

Button Clear
Click this button to clear the Spot selection.

No Spot Selected/Selected Spot

Position X: Y: Z: [um]
Display of the Spot position.

Diameter [um]
The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

Time Index
The number indicates the current time point in the series for the selected object.

Spots

In Imaris it its possible to merge two or several Spots items into one Spots item.

Create Track from Selection... Button
Clicking this button creates a track for the selected Spot(s).

- Set the **Mouse selects** option to **Spots**.
- Add new Spot with Shift+ left click or select the already created one.
- Clicking on the **Create Track from Selection** button brings up the Create track from Selection Window

Create track from Selection Window

1. Select the Source channel

2. Spot Detection
Estimated Diameter
This is the diameter of the inserted Spots. Spots with a diameter smaller than this are not detected.
There are two options for a Surface creation Threshold:
Background Subtraction
If checked, the background will be removed prior to spot detection.

3.Tracking algorithm
The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Spots. Based on these predicted positions the best matches to candidate Spots can be determined
There are two algorithms to choose from:
Brownian Motion
This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernable pattern).
The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.
Autoregressive Motion
This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction . The user-specified maximum distance value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

4. Max Distance
The maximum search distance that a tracked object are looked for is specified with the Max Distance value.
The parameter Maximum Distance disallows connections between a Spot and a candidate match if the distance between the predicted future position of the Spot and the candidate position exceeds the maximum distance.

5. Max Gap Size
An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

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Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size file.

The **Max Gap Size** defines the maximum number of the consecutive time points witch are allowed to be missing in order to a track to be continues.

6. End Time Point
The parameter specifies the last time point for the tracking algorithm implementation.

OK Button
Cancel Button

Button **Merge ...**
This button is only available if you have two or more Spots items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Spots X" displays (see below).

Window: Merge selected Objects to "Spots X"

Check the boxes in front of the desired Spots items or use the button **Select All**.

Manual Tracking

The Manual Tracking option can either be used when automatic tracking algorithms require modifications and adjustments, or to create an entire track from scratch.

The manual tracking option allows you to track objects within a time-resolved data set by manually specifying the object location in each time point. Essentially, the manual tracking is done by clicking on the structure in the image, which serves as a visual reference (template).

This option can be activated by clicking in the box Auto-connect to selected spot.

To start manual tracking, move the time slider to the first time point of the feature you want to track. Then use the pointer to Shift and then left click on the feature in the dataset. After each mouse click, the time slider advances one frame, allowing you to see the subsequent image of the temporal stack. To continue manual tracking, keep clicking on the structure on the image. As you add more manual tracking points by clicking within the viewing area, a track is created allowing you to follow the moving features, frame by frame and from point to point, until you've built a complete motion path.

Please note Manual tracking behaviour is governed by the Selection mode under Track Edit tab.

Auto-connect to the selected spot box

Select the option to add all selected or added objects to one track and advance to next time point when a new object is added or an existing is selected.

Please note: Objects are always added at the current time point (t).

The result of Manual tracking can be very different depending on which selection is chosen in the Track Edit tab.

If the option **Track** is selected, a connection is established between added object at time point t and the last added object of the selected track at time point t-1.
If the option **Spots** is selected, a connection is established between the selected object at the immediately preceding time point (t-1) and the newly added object at time point t.

Please note: If there is no the immediately preceding time point, a new track will be constructed.

The box on the drawing plane represents the created object diameter. The diameter of the object can be changed, during the manual tracking, by using the mouse wheel. Use the mouse wheel to increase/decrease the radius of the created object.

Button **Clear All**
Use this button to clear the Spots selection.

Button **OK**
If you want to merge the Spots click on **OK**.

Button **Cancel**
If you do not want to merge the Spots click on **Cancel**.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

See also:
Surpass View - Overview - Camera Toolbar (Pointer Navigate/Select)

14.12.5 Tab Edit Tracks

Mouse Selects

This option allows you to select an object within an image according to its object type by a single mouse click. To edit the properties of multiple objects simultaneously, select the required object type.
There are two options:
Spots
Track

Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.
The first row of the track-editing table displays the timeline. Each number represents a time point in the series.
The second row of the track-editing table displays the **object name**.
Multiple object types can be tracked easily since every object type appears in different colors.

Object ID

If the mouse pointer is on the object, a tooltip appears that identifies the object ID. The ID numbers of the objects are sequentially ordered column-wise from the top to the bottom of the table. The object maintains its unique ID number independent of the track being allocated to.
If the mouse pointer is on the connection, a tooltip appears that identifies the track ID number.

Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.
In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
For multiple selections press Ctrl+click (Mac: Command+click).

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Visualization options

Zoom
Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.
Using the mouse wheel will have the same affect.
Pan
To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

Time indicator
The first row of the track-editing table displays the timeline. Each number represents a time point in the series. The current time point is indicated by vertical line highlighted with in current selection color.

To modify the time point, do one of the following:
1. Moving the slider in the Time bar modifies its position in time and changes the time point in the track editor.
2. Clicking on the time point within the track-editing table automatically moves the time line indicator to the corresponding point.
Double clicking on the time indicator, fixes the position of the current time point at the center of the track-editing table, regardless of the selected zoom or pan options)

Editing

Track Editor has several features that help you modify your tracks.

Add tracks

To set the starting point of a new connection first you have to select the object.
Shift+left-click followed by a mouse movement on the object creates the new connection.
To establish a connection, drag the edge to the target object. If valid, the target object is highlighted (red).
Release the mouse button and the line segment clips automatically onto the new object, creating a new connection between the two objects.

Please note: The new connection is only possible between the objects of the same type. If you release the mouse button without being on a target object, the edge will disappear and a new connection will not be established.

A connection between objects of the same time point is also invalid.

Delete Tracks Edges

Tracks
Select the track and press backspace to delete the whole track.

Connections
To delete the connection, select the connection and press shift-click. The selected connection is deleted.

The process of deleting the connection splits the track. The first track part remains with the same ID number and stops at the time point of the deletion. The second part consists of the remaining deleted objects and the connections between them. It is automatically shifted to the bottom of the track-editing table and assigned a new track ID number.

Connect unconnected components

Manually added, or previously untracked objects are displayed as separated circles. The procedure of creating new connections and connecting the objects is unchanged.

Show Selection (build working set)

This option allows you to work on subset of tracks.

Connect Track Button

Click this button to connect two existing tracks. Select two Spots in two different tracks at the subsequent time points (just one Spot per time point) and click on the button Connect Track. The connection is automatically created between all selected objects.

Disconnect Track Button

Select a object and click on the button Disconnect Track. The connection from the selected object to the next object in time sequence is deleted.

Correct Drift

If you have an object drift (e.g. a cell drift) during the image acquisition you can use the Correct Drift button to correct the drift for the whole data set.

The ImarisTrack tracks all objects in an image, even the objects that should be stationary.
The Correct Drift function gives the user the opportunity:

1) to specify the tracks related to the stationary objects
2) to assign these objects as stationary objects and
3) to adjust the position of the rest of the image objects based on this selection.

As a result of drift correction, the entire image is transformed in such a way that the selected objects remain stationary and within the new image each objects' position (changes) based on the correct draft algorithm. Within the new image, the true movement of the objects can be then detected relative to the stationary objects.

In the accordance with the selected for **Result DataSets** options. the new image coordinates are calculated and displayed.

To make the Drift Correction on the data set the following steps are completed:

- Select the tracks associated with the stationary objects
- Select the algorithm for drift correction
- Select the option for the Result data sets
- Verify the selection and click on the OK button.

Please note: The drift correction changes the data set.

Based on the provided the track sub-selection the algorithm determines the new objects position for all time points.
If the tracks are selected, the algorithm will be performed on the sub selection. Otherwise, every track of the image is considered for calculations.

Algorithms

The Drift Correction is calculated based on the two algorithms, Translation and translation and rotation.

Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

Translation and rotation

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Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

Please note: The number of the selected tracks affects the algorithm calculation.

Selection of a single track gives a complete description of a translation.
Selection of a two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).
A better fit between the time points is achieved when three or more tracks are selected.

Result DataSet size

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.
Select the Result data set option to determine which new image part will be displayed.

Crop largest common region

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

New size equal to current size

The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

Include entire result

The final image will be the size of the combined area of the original and corrected image.
Selecting the appropriate Result dataset size may speed up the data visualization. This is especially important with (with large data sets.)

Correct spots positions

Activate this parameter and the spots are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.12.6 Tab Filter

Please note: Before using you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the

selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated. Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode.

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Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of the selected objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Spots) is displayed at the bottom of the threshold histogram.

Button **Duplicate Selection to New Spots**
Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

See also:
Surpass View - Overview - Surpass Tree

14.12.7 Tab Statistics

Spots statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics -Spots. The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.
Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:
a) Overall
b) Detailed
c) Selection
In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.
To sort the columns click on the arrow in column heading. Click again to reverse the sort.

Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Detailed Tab

A pull down list offers three types of **Detailed** statistical calculations:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.
Object Statistical Annotation option is available for Specific values parameters.

Average values
The Average table contains values determined on the basis of all values within a set of the selected variable.
Columns are labeled as follows:
Min (Minimum)- the smallest value within a set of values of the selected variable
Max (Maximum)- the largest value within a set of values of the selected variable
Mean – value of the sum of all values divided by number of values within a set
Std Dev (Standard deviation)- a measure of the values distribution around the mean values

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Median- the middle value of the sorted set of variables
Sum- the sum of all values within a variable set
Count- number of values within a set
Unit- variable unit
Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Statistical variable classification:

1. Statistical variables associated with objects (Cells, Nucleus, Vesicles -volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables the Average values are displayed per time point. The changing the time points, statistical variable values and images are consequently changed.
2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement length, track intensity) are not time dependent.

All values
If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.
Object Statistical Annotation option is available for Specific values parameters.

Selection Tab

A pull down list offers three types of statistical calculations from a Selection:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.
Object Statistical Annotation option is available for Specific values parameters.

Average values
The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

All values
If the option All values is selected, all statistical values will be displayed for the selected image element.
Object Statistical Annotation option is available for Specific values parameters.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Buttons Overview

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.
Click on the button **Settings** and you switch to the window Preferences - Statistics - Spots.

Button **Search** - Search by object ID.
Type in the object ID in the data field. Click on the button **Search**.
- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Spots** - Duplicate Selection to new Spots.
Click on this button and all selected elements are copied to a new Spots object. The new Spots appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation**
Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** or **All Values** are selected.

Button **Export** - Export Statistics on Tab Display to File.
Only the statistics that are shown within the current tab will be exported.

Button **Export** - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.
All of the statistics (that are enabled in the statistics settings) will be exported.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:
Menu Edit - Preferences... - Statistics - Spots

14.12.7.1 Statistical Annotations

Statistical annotations provides the opportunity to view and annotate image objects with the statistical data values. Using this function, the individual objects in an image are labeled with the specified statistical values and the statistical data is displayed directly in the viewing area

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Imaris objects can be labeled with a set of any number of the user-defined statistical values.
The Statistical Annotation button provides a convenient interface in order to label the object with the statistical data. Click this button and an object statistical annotation will be automatically generated in the viewing area. The selected object is annotated for all time points with the specified statistical values.

How to add Statistical Annotation

In the Statistical tab choose the **Detailed** or **Selection** Tab.
In the viewing area select the object or group of objects. Change to the pointer mode Select and click on an image object that has been detected/segmented. For multiple selections press Ctrl+click (Mac: Command-click)
In the **Detailed** or **Selection Tab** a pull down list offers **Specific Values** or **All values** as types of statistical parameters.
Select the statistical value associated with the particular object type you are working with.
Press the Statistical Annotation button .
In the viewing area the object label appears showing selected statistical values as a statistical annotation.

The statistical annotation shows by default the name of the selected statistical parameter and its value.
The color white displays the text of annotations associated with statistical values.

For a single object, all selected statistical parameters are placed in the same annotation box. Once the object is labeled, selecting the additional statistical parameter updates the object statistical annotation; the new parameter and its value is added in the viewing area.
If the image is a time series, the statistical annotation will be visible in the viewing area for all time points. Selecting a statistical variable that changes over time creates an statistical annotation that consequently changes with every time point.
The statistical annotation displays values always for the current time point.

The InPress options will allow you to modify and specify the contents and format of the object labels.
To modify and adjust the statistical label click on the Icon InPress , press Ctrl + Shift + P, or in the menu bar select File – InPress.

14.12.8 Tab Color

Tab Color edit, manage and assign colors material to the Spots or Tracks.

Object Type

Choice of the Object type is presented in a drop down window. Select either Point (for Spots) or Tracks and depending on the chosen object type selection of the variables is listed.

For **Object Type Points** two color types are available:
Base
Statistics Coded

For **Object Type Tracks** three color types are available:
Base
Statistics Coded
Time Mapped

14.12.8.1 Color Type-Base

Base
Assign the Spots/Track color via the Color Editor. Tab Color edit, manage and assign material colors of a Spots/Track.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.12.8.2 Color Type-Statistics Coded

Statistics Coded

Depending on the chosen object type the Statistical variable selection is listed in the drop down window. For example, if Point is chosen as the object type, available Statistical data will be Point linked variable. Similarly, variables for Tracks will be listed, if the object type is selected to be Tracks.

Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area. The upper one is the colorbar range for Points and lower one is the colorbar for the Tracks.

Show Colorbar
Check the box to see the statistics colorbar in the viewing area.

Show Title
Check this box to see the title in the statistics colorbar.

Show Range
Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**
Click the **Reset** button to set the image back to the original values.

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Button **Load** …
Click on the button **Load** to display the Import Color Table File color window. Enter the path and the requested File name and/or File type and click Open. The selected file is loaded and displayed.

Colormap range

Colormap range-specify the limits for the colorbar.
Min - lower color limit
Max - upper color limit
Button **Auto**
Click the button **Auto** and the system detects the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

14.12.8.3 Color Type-Time Mapped

Time Mapped
This option is only available when Tracks (see above) is selected as Object Type. The color range for the Tracks goes from blue to white to the range of time points.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

14.12.9 Mouse & Keyboard PC

Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Left-click
Select multiple Spots	Ctrl + left-click
Add new Spot	Shift + left-click
Delete Spot	Left-click to select, then click on the the button Delete
Change Spot diameter	Mouse wheel

See also:
Addendum - Mouse & Keyboard PC

14.12.1(Mouse & Keyboard Mac

Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Click
Select multiple Spots	Command + click
Add new Spot	Shift + click
Delete Spot	Click to select, then click on the the button Delete
Change Spot diameter	Mouse wheel

See also:
Addendum - Mouse & Keyboard Mac

14.13 Surfaces

The Surfaces visualization is a computer-generated representation of a specified gray value range in the data set. It creates an artificial solid object in order to visualize the range of interest of a volume object.

How to Add a new item Surfaces?

- Select menu Surpass - Surfaces.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Surfaces - Properties.

See also:
Surpass View - Overview - Surpass Tree
Surpass View - Surfaces - Creation Wizard

14.13.1 Creation Wizard

The Creation Wizard allows to go forth and back until the optimal parameters are found.
In the first window of the Surface Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.
The most convenient way to apply the same creation parameters to different data sets is to use the **Load Parameters** option.

In the first window of the Creation Wizard you find the algorithm settings. Here you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection, use the **Seeded Region Growing** for the Surfaces detection or to **Skip automatic creation** and start Contour Surface drawing .

If you do not want to use the automatic Surface creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the manual Surface creation.

For further details please refer to chapter Algorithm, Region of Interest, Source Channel, Threshold, Split touching Objects (Region Growing), Classify Seed Points, Classify Surfaces, Tracking, Edit Surfaces, Classify Tracks, and Complete ROI.

14.13.1.1 Algorithm

If you do not want to use the automatic Surface creation wizard select the **Skip automatic creation, edit manually** button.

Skip automatic creation, edit manually Button
Terminates the creation wizard and activates the manual Surface creation.

Load Parameters

This option lets you use the identical creation parameters for multiple datasets.
The option to Load Parameters is only available if the set of the creation parameters has already been saved under the Creation tab using the Remember Parameters button.
Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are

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automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Load Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

If you do not want to use the automatic Cell creation wizard select the **Skip automatic creation, edit manually** button. If you do not want to use the automatic Cells creation wizard select the **Skip automatic creation, edit manually** button.

Algorithm

Segment only a Region of Interest
A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.
Check this box if you want to segment only a Region of Interest. If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

Process entire Image finally
Process entire Image finally this option appears only, if Segment only a Region of Interest is checked.
Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters (Complete ROI). Uncheck this box and only the region of interest is processed.

Track Surfaces (over Time)
Check this box if you want to track the resulting Surfaces over time, for details please refer to tab Tracking.

Some Examples

Intensity Threshold	Structure of the Creation Wizard is: • 1/4 Algorithm • 2/4 Source Channel • 3/4 Threshold • 4/4 Classify Surfaces
Intensity Threshold + ROI	Structure of the Creation Wizard is: • 1/5 Algorithm • 2/5 Region of Interest • 3/5 Source Channel • 4/5 Threshold • 5/5 Classify Surfaces
Seeded Region Growing	Structure of the Creation Wizard is: • 1/7 Algorithm • 2/7 Source Channel • 3/7 Threshold • 4/7 Split touching Objects (Region Growing) • 5/7 Region Seed Points • 6/7 Classify Seed Points • 7/7 Classify Surfaces
Intensity Threshold + Tracking	Structure of the Creation Wizard is: • 1/7 Algorithm • 2/7 Source Channel • 3/7 Threshold

	• 4/7 Classify Surfaces • 5/7 Edit Surfaces • 6/7 Tacking • 7/7 Classify Tracks
ROI + Seeded Region Growing	Structure of the Creation Wizard is: • 1/11 Algorithm • 2/11 Region of Interest • 3/11 Source Channel • 4/11 Threshold • 5/11 Split Touching Objects (Region Growing) • 6/11 Region Seed Points • 7/11 Classify Seed Points • 8/11 Classify Surfaces • 9/11 Edit Surfaces • 10/11 Tacking • 11/11 Classify Tracks
Seeded Region Growing + Tracking	Structure of the Creation Wizard is: • 1/10 Algorithm • 2/10 Source Channel • 3/10 Threshold • 4/10 Classify Surfaces • 5/10 Split touching Objects (Region Growing) • 6/10 Region Seed Points • 6/10 Classify Seed Points • 7/10 Edit Surfaces • 8/10 Tacking • 9/10 Classify Tracks

Surfaces Wizard Buttons

Button **Back**
This button is grayed in the first window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.2 Region of Interest

Region of Interest 1

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

X, Y, Z, Time
Min, Max, Size
Minimum, maximum and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields. Alternatively the location and size of the ROI can be adjusted directly in the viewing area.

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Size of the ROI:
1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X ,Y and Z.
Location of the ROI:
1.Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.
The numbers in the value fields are updated automatically.

Memory Usage:
Display of the memory usage for the image size within the ROI.

Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

Button **Add**
Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

Button **Delete**
Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

Total Memory Usage:
Display of the memory usage for the image size within the ROI.

Button **Cancel**
If you do not want to create Surface click on **Cancel**.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

See also:
Surpass View - Overview - Camera Toolbar (change to pointer mode Select)

14.13.1.3 Source Channel

Source Channel

Select the respective source channel, from which the Surfaces are to be computed.

Smooth

Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.
Smoothing may require a great deal of memory if the dataset is very large. Uncheck the check box or use

smaller ROI if your system does not have enough memory.
If the option Smooth is selected then the **Surface Area Detailed Level** value should be determined. Use either the automatically given value or type in a new value in the data field.

Threshold

There are two options for Surface creation Threshold:

Absolute Intensity
Background Subtraction (Local Contrast)

Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.
The width of the Gaussian filter is calculated by subtracting the diameter of the **largest sphere that fits into the Object** from the intensity of the original channel.
If the option Background Subtraction is selected then the **Sphere Diameter** should be determined.

Sphere Diameter

The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of **Sphere Diameter** should be slightly larger than the diameter of the largest object expected to be found in the dataset.
If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.4 Threshold

Threshold

Activating/deactivating the threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the threshold option is activated (the green button), the corresponding A/M button turns black.

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After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

General

Automatic
The initial threshold value is automatically computed using an algorithm based on [T.W. Ridler and S. Calvard, "Picture thresholding using an iterative selection method", IEEE Trans. System, Man and Cybernetics, SMC-8 (1978) 630-632].

Manual
Define the requested threshold by clicking into the histogram or by entering the value directly in the data field.

Seeded Region Growing

If you want to apply the seeded region growing for the Surfaces creation check the respective option on the first window of the Surfaces Creation Wizard, tab Algorithm Settings. During the region growing process around each seed point the region grows until a border stops the growing. The threshold you set above is the border of the region growing.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.5 Split touching Objects (Region Growing)

This tab only appears, if **Split touching Objects (Region Growing)** is checked (Algorithm Settings).
This option supports the separation of two or more objects that are in the Threshold step has identified as one. The region method for segmentation in Imaris starts with defined seed points. You use Surfaces as seed points for the region growing that is performed in the next steps. During the region growing process around each seed point the region grows until a border stops the growing.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.6 Region Seed Points

Seed Detection

Estimated Diameter
Type in the estimated diameter of a seed point.

14.13.1.7 Classify Seed Points

The Seed Points filtering is incorporated into the creation process. This tab only appears, if **Split touching Objects (Region Growing)** is checked (Algorithm Settings).

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks,

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Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).

The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within

threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.8 Classify Surfaces

The Surfaces filtering is incorporated into the creation process.

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

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Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated. Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

Automatic

The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.9 Edit Surfaces

Here you find the manual editing tools for the Surfaces.

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Edit Mode

Add/Delete
This activates a semi-automatic surface segmentation (see below). Single objects can be added by one mouse click. If you select **Add/Delete** as edit mode, the Add/Delete Properties are available (see below).

Cut
This activates the Surface Scissors function (see below). You can manually split two touching surface objects. If you select **Cut** as edit mode, the Cut Properties are available (see below).

Autodepth Draw

The auto depth function will automatically calculate the Surface depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

Intersect with:

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all visible Channels
specific Channel
solid Objects
Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

Add/Delete Properties

The Add/Delete Properties are only available, if you select **Add/Delete** as edit mode (see above).

There are two different techniques available:

Marching Cubes
Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).
- Position the autodepth cursor to the center of the object.
- Adjust the size of the box to the approximate size of the object (use the mouse wheel).
- Shift + click to create the new object.

Magic Wand
Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

- Position the autodepth cursor to the border of the object (the size of the box does not influence the result).
- Shift + click to create the new object.

Process Selection

Button **Clear**
Click this button to clear the Surfaces selection.

Button **Duplicate**
Click this button and all selected Surfaces appear in a new group in the Surpass Tree (Surfaces Selection).

Button **Delete**
Select a Surface and click on this button to delete the Surfaces.

Duplicate to all Timepoints
If you check this box the selection is not only active for the current time point but for all time points in which the object appears.

	PC:	Mac:
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

Scissors Properties

Use the Surface Scissors to manually split two touching Surface objects.

Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.

- Change to the pointer mode Select.
- Select as Edit Mode the mode Cut (see above).
- Shift + click on the Surface object.
- A colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular).
- Click on the button **Cut Surface** (see below) and the object splits into two individual Surface objects with two different colors.

Button **Cut**
Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

Button **Clear**
Click on this button to clear the selection.

Close Borders
Check this box to add a surface along the cutting line and close the surface.

Mask Properties

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask All...**
Button **Mask Selection ...**
Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

Surfaces

In Imaris it its possible to merge two or several Surfaces items into one Surfaces item.

Button **Merge ...**
This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

Window: Mask Channel

Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

Duplicate Channel before applying Mask
When this option is selected, the channel will be duplicated before applying Mask.

Mask Settings

Random Color Map
Constant Inside/Outside
Define, if you want to use a random color map or if you want to set a constant inside/outside.

Set Voxels outside Surface to:
The default value is 0.000, no voxels outside the region of interest are displayed.

Set Voxels inside Surface to:

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If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

Apply to all Time Points
Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button **OK**
If you want to apply the mask click on **OK**.

Button **Cancel**
If you do not want to apply the mask click on **Cancel**.

Window: Merge selected Objects to "Surfaces X"

Check the boxes in front of the desired Surfaces or use the button **Select All**.

Button **Clear All**
Use this button to clear the Surfaces selection.

Button **OK**
If you want to merge the Surfaces click on **OK**.

Button **Cancel**
If you do not want to merge the Surfaces click on **Cancel**.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.1 Tracking

Tracking allows objects to be traced throughout sequential frames of a time datasets, so each object maintains a unique identity in the output measurements.
This tab only appears, if **Track Surfaces (over Time)** is checked (Algorithm Settings).

Algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Surfaces. Based on these predicted positions the best matches to candidate Surfaces can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while Autoregressive Expert mode combines changes in position and intensity to compute total cost.

Brownian Motion
This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Surface perform similar to a Brownian motion (the random movement of Surface with very frequent changes in direction and no discernable pattern).
The only prediction applied is that the Surface does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

Autoregressive Motion
This algorithm models the motion of each Surface as an autoregressive AR1 process. This model is appropriate if your Surface perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Surface will move again the same distance and in the same direction. The user-specified maximum distance value is the distance in which the Surface is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

Autoregressive Motion Expert
This algorithm models the motion of each Surface as an autoregressive process. This model is appropriate if your Surfaces perform any kind of continuous motion. In the expert mode the tracks are predicted by the additionally established algorithm called Intensity Weight (see below).

Connected Components
For this algorithm, Surfaces in adjacent time-points are considered connected if the Surface spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. The object with the greatest amount of overlap will be assigned the same ID.
This is the only algorithm that automatically handles lineage (spots that split or merge). It will only work with images that have excellent time resolution, because the Surface cannot ever jump a distance larger than its own size.

Parameters

Max Distance
The maximum search distance that a tracked object are looked for is specified with the Max Distance value. This parameter disallows connections between a Surface and a candidate match if the distance between the predicted future position of the Surface and the candidate position exceeds the maximum distance.
The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size file.

The **Max Gap Size** defines the maximum number of the consecutive time points witch are allowed to be missing in order to a track to be continues.

The parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see above).

Fill the gaps with all detected objects

If this option is selected the algorithm performs the optimization of the object expected position for the

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particular time point. The track is generated by connecting objects assuming their optimized expected positions.

Intensity Weight
Tracks are computed by minimization of a cost function that combines distance based and intensity based costs. "Intensity Weight" is the factor by which IntensityCost is taken into account:
TotalCost = sum_(all connections) DistanceCost + IntensityWeith * IntensityCost
The parameter is only available if you select Autoregressive Motion Expert (see above).

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.1 Classify Tracks

The Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Surfaces (over Time)** is checked (Algorithm Settings).

A filtering allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated. Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Higher Threshold

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Automatic
The initial threshold value is automatically computed.

Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**
Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

Button **Cancel**
If you do not want to create Surfaces click on **Cancel**.

14.13.1.1 Complete ROI

This tab only appears, if **Process entire Image finally** is checked (Algorithm Settings).

Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

Surfaces Wizard Buttons

Button **Back**
With **Back** you return to the previous window of the Creation Wizard.

Button **Next**
This button is grayed in the last window of the Creation Wizard.

Button **Finish**
Click on **Finish** to end the calculation and display the results in the viewing area.

Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

14.13.2 Tab Settings

Surfaces Style/Quality

Surface
Select this option and the created Surfaces is displayed in the viewing area.

Center Point
This option displays the Surfaces as center points.

Off
Select this option to disable the visualization of the Surfaces.

Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

Off
Select this option to disable the visualization of the Tracks.

Line
This option displays the tracks connections dawn as lines.

Cylinder
This option displays the tracks connections dawn as lines.

Width
Diameter
Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

Render Quality
When you select Cylinder (see above) the Render Quality is adjustable using the slider.

Tracks Path

Displacement
Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

Color Editor
Select a desired color for the displacement arrow.

Time Color
Selecting Time Color draws lines, cylinders or truncated cones in colors with a range from blue to white and dark to bright corresponding to the range of time points. Alternatively it is possible to assign a single color via the Color Editor. Deselect Time Color and click on the Color symbol to open the Color Editor.

Color Editor
Select a desired color for the Track display.

Radius Scale
Edit field for the displacement vector radius.

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Dragon Tail
Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

Slider
Adjust the number of time points using the slider.

See also:
Surpass View - Surfaces - Creation Wizard

14.13.3 Tab Creation

Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list on which step of the Surfaces Creation Wizard you want to re-enter the creation process.

For details please refer to chapter:
Menu Surpass - Surfaces - Algorithm
Menu Surpass - Surfaces - Tracking

Rebuild Objects Button
Click on this button to start the rebuild.

Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Surfaces computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

Remember Parameters Button

The Remember Parameters button allows you to save the complete set of the creation parameters and the relevant settings shown in the list.
Storing the parameters allows them to be easily reused, modified, or exchanged.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.
When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/Creation Parameters.

Click on the Remember Parameters Button opens the Remember Creation Parameter window.

Remember Creation Parameter

Enter a name for the Creation Parameter set. This name identifies the Creation Parameter item in the Creation Parameter list used in the Edit/Preference/Creation Parameters and would be used as default name in the subsequent creation wizard algorithms.

Add to Batch Button
Add to Batch button places the command into Batch coordinator with all adjusted parameter values.

14.13.4 Tab Draw

The Draw tab allows the user to semi-automatically create further Surface objects.
The Draw tab has two sub tabs:

Add/Delete
Contour

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.13.4.1 Add/Delete

Add/Delete
This activates a semi-automatic surface segmentation.
Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

Intersect with:
all visible Channels
specific Channel
solid Objects
Select as reference for the Surface depth the first visible channel, all visible channels, or other solid objects.

Add Surface using:

There are two different techniques available for adding the Surface Objects:

Marching Cubes
Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).
- Position the cursor to the center of the object.
- Adjust the size of the box to the approximate size of the object (use the mouse wheel).
- Shift + click to create the new object.

Magic Wand
Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

- Position the cursor to the border of the object (the size of the box does not influence the result).
- Shift + click to create the new object.

14.13.4.2 Contour Surface

The Contour Surface allows you to extract a 3D object by manually drawing the object contours on 2D slices. You can employ this method whenever simple threshold does not yield individual structures (e.g. touching cells in confocal microscopy, complex tissue recorded by transmission microscopy).

It is recommended to uncheck any other object in the Surpass Tree before drawing the contour, so that only the contour is visible in the viewing area.

The Contour Tab has three sub-tabs:
Board
Mode
Autofit

14.13.4.3 Board

A drawing plane is displayed in the viewing area.

Orientation

This option defines the orientation of the drawing plane. Once orientation of the drawing plane is selected, the

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drawing planes of all contours are set in the same position. By default the orientation is set to be in the XY plane-z-direction.
Choose the **YZ**-plane to draw in the x-direction. Choose the **XZ**-plane to draw in the y-direction. Choose the **XY**-plane to draw in the z-direction.

Visibility

This option defines the Visibility of previously drawn contour lines.

None
The contour currently being drawn is made visible.
Next
The contour(s) next to the contour currently drawn is/are shown.
All
All contours are shown.

Resolution

This option controls the smoothness and complexity of the surface created by the contour lines. It can be used to reduce the number of triangles in a surface object according to a user-defined value. There are two options:
Automatic
The Automatic resolution depends on the contour line vertex spacing.
Manual
The manual resolution is controlled with the slider. Moving the slider to the left reduces the resolution, moving it to the right increase resolution.
Setting the Resolution to 100% corresponds to the data set resolution. Setting the resolution to 1% reduces the voxel resolution to 1% of original data set.

Preserve feature

If the box Preserve feature is selected, a Surface calculation is then performed using anisotropic Gaussian filtering. This feature aims to reduce image noise without removing significant parts of the image content, ensuring that thin and sharp features are not absent from the created surface. The filter size is calculated adaptively and separately for each plane. It produces a family of contour lines, and each resulting line is a combination between the original image and a filter that depends on the local content of the original image. As a consequence, in the created Surface noise is removed, and significant parts of the image content (like elongated and narrow features) are preserved.

If the Preserve feature is not selected an isotropic Gaussian filter is used for the Surface creation. The smoothing is made along the structures, resulting in consistently smooth Surface.

Please note: As a result of applying isotropic Gaussian filter, thin and sharp Surface features may fail to be created.

Selection

The following button actions apply to the previously created contour lines.

Button Cut
To cut a contour line in a plane click on the button Copy.

Button Copy
To copy a contour line in a plane click on the button Copy.

Button Paste
To paste a contour line in click on the button Paste.

Button Delete
To delete a contour line click on the button Delete

Button Smooth
To smooth a contour line edge click on the button Smooth.

Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

Slice (data field)
The respective slice number is displayed in the number field.
The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and than simply click at the drawing plane and drag it to a new position.

Lock button
This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.
Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.
The L key allows the switch between locked and unlocked orthogonal slicer.
Please refer to the chapter Shortcuts Overview in the Reference Manual for a detailed list of shortcuts for manual surface creation.

Draw button

Click on this button to alter **between** the **two** Drawing Modes: **Active** and **Inactive**

Drawing Modes

In the contour line, creations there are two different drawing modes: **Active** and **Inactive**.
Active
The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines.
Inactive
In the inactive drawing mode, the existing contour lines can be modified or deleted.
You can select the respective mode by using the **Draw button** or **Ctrl+space key**.
Press the **Draw button** or **Ctrl+space key** to alter Drawing mode between **Active** and **Inactive** mode.

The pointer's appearance on the screen depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

Create Surface button

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

14.13.4.4 Mode

Drawing Modes

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In the contour line, creations there are two different drawing modes: **Active** and **Inactive**.

Active

The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines.

Inactive

In the inactive drawing mode, the existing contour lines can be modified or deleted.

You can select the respective mode by using the **Draw button** or **Ctrl+Space key**.

Press the **Draw** button or **Ctrl+Space key** to alter Drawing mode between **Active** and **Inactive** mode.

The pointer's appearance on the screen depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

Please refer to the section 'Shortcuts Overview' for a listing of the respective shortcuts.

Several segmentation tools are provided allowing you to select areas in the current slice for contour line drawing.

Select one of the shape object tools in the Drawing Mode. Left clicking on a tool icon activates a drawing tool. Once a drawing style tool is selected you may adjust the specific parameters for that drawing mode.

Once a segmentation tool is activated and adjusted, activate the drawing mode by changing the pointer mode to Select (press the ESC-key) and then once press the **Draw button** or **Ctrl+space** key. The Drawing mode is activated.

By default, contour lines are drawn in a transparent gray color. The color of the contour lines can be modified using a dialog under the Color Tab.

Click

Clicking within the image inserts a point, a so-called vertex. You can now define subsequent vertices by clicking with the left mouse button. Successive points will be connected with straight-line segments.

Time

Click once on the contour and then move the mouse along the contour. The number of vertices depends on the time interval (ms), specified in the corresponding field. The interval can be changed by manually specifying the required value. This drawing style allows you to draw a structure that contains fine details.

Insert a vertex after ms

The number specifies the time interval (in ms) between two inserted vertices.

Distance

Click once on the contour and then simply move the mouse along the contour. The number of vertices depends on the distance interval (um), which is specified in the corresponding field. To change the interval, specify the required value in the object properties area. This drawing style is recommended if the structure is smooth.

Vertex spacing um

The number specifies the distance interval (in um) between inserting two inserted vertices.

Circle

The Circle drawing tool inserts a circular contour line. The Circle tool always draws a contour line from the center; that is, the place you initially click will be the center point of the contour line. To change the circle radius specify the required value in the object properties area. In the parameters you can also specify the number of contour vertices to be inserted. To create the contour line, move the mouse pointer over the orthogonal slicer plane. A preview outline of the circle is then displayed. To create a contour line, click with the left mouse button on the desired position.

Radius um

Defines the radius of the circle in um.

Scrolling the mouse wheel can also change the radius of circle.

Number of vertices
Defines the number of vertices in the circle line.

Isoline
The Isoline drawing tool generates contours at a fixed iso-value so the geometrical features of the contour line takes into account the image's intensity values. Connecting the points with a constant intensity value forms the contour line.
The Isoline drawing mode selects the maximum intensity iso-value under the mouse pointer and creates a contour line by tracing points of uniform intensity. Clicking with the left mouse button on the orthogonal slicer plane selects the largest connected area that contains the all voxel with the same intensity values. The current iso-value under the mouse pointer is displayed in the parameter field Intensity and is updated automatically as you move the mouse. To create a contour line, click with the left mouse button on the desired position.

Reduce density to numerical field %
Defines the density of inserted vertex in relation to the data set.
A density of 100% inserts a contour vertex at each pixel of the data set.
A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Intensity
Displays the value of the voxel intensity under the mouse pointer.

Magic wand
The Magic wand tool creates a contour line by selecting the largest connected area that contains the maximum intensity voxels and all voxels within a user-defined tolerance range. The Tolerance range defines the tool's sensitivity; the higher value you set, than wider image area that is selected. The Tolerance range can be specified in a box in the Parameter panel.
Move the mouse pointer over the orthogonal slicer plane and a preview of the contour line is displayed. The Intensity value in the Parameter panel is updated as the mouse pointer is moved across over the orthogonal slicer plane. To create a contour line, click with the left mouse button on the desired position.
The Intensity with the corresponding Tolerance value is stored in the parameter section of the label field. This makes it possible to easily correct a selection at a later time.
It is possible to modify the range even after the seed voxel has been selected.

Reduces the density to numerical field %
Defines the density of the inserted vertex in relation to the data set.
A density of 100% inserts contour vertex at each pixel of the data set.
A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Tolerance
Defines the range of intensity limits in percentages.
The tolerance 0% selects pixels with exactly the same intensity value as the point under the mouse pointer. The tolerance value of 100% selects all intensities within the intensity range of the data set. Values in between are interpolated linearly. The tolerance can also be changed incrementally by scrolling the mouse wheel.

Intensity
Displays the value of the voxel intensity under the mouse pointer and the tolerance range.

Detects holes box
This option enables you to create a contour line that contains holes of selected areas. If the Detects holes box is selected the line is generated around holes, outlining the hole. If the box in unchecked, the outer line of the selected region is drawn and the holes are not visible.

Channel Selection

All Visible Channel

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Specific Channel
Select the respective source channel, from which the Contour surface is to be computed.

Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

Slice (data field)
The respective slice number is displayed in the number field.
The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and than simply click at the drawing plane and drag it to a new position.

Lock button
This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.
Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.
The L key allows the switch between locked and unlocked orthogonal slicer.
Please refer to the chapter Shortcuts Overview in the Reference Manual for a detailed list of shortcuts for manual surface creation.

Draw button
Click on this button to alter **between** the **two** Drawing Modes: **Active** and **Inactive.**

Create Surface button
To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

14.13.4.5 Autofit

Autofit is a method that can help the user automate a manual segmentation.
Autofit is a deformable contour model used to create and optimize the contour line segmentation, so that the localization and accuracy of the segmented object is improved.
It is a semi-automatic, user interactive detection, robust against noise in the image. The contour line creation procedure consists of two steps: initial user segmentation and refinement by the Autofit algorithm.
In the first step the model requires the user input, an initial curve (rough outline) closed to the boundaries of the object to be segmented.
The next step is to perform an Autofit algorithm. The algorithm then tries to deform this initial shape so that it fits onto the contours of the image. The Autofit algorithm performs an iterative optimization process where the sum of external and internal energies along the contour line approach a local minimum.
The external energy is computed from the original image intensity gradients and it should be minimal when the contour line is located at an object boundary.
The internal energy is based on the shape of the contour line. The Autofit algorithm simulates the contour line as an elastic rubber band and the internal energy is defined by the physical constraints that describe the contour. It should be minimal when it meets some regularity and smoothness constraints regarding the shape of the line (curvature is minimized and contour vertices are encouraged to be equidistant).
The outcome of the Autofit algorithm is an optimized line, a good fit between the external forces, which aims at the object boundaries, and deformable internal forces that targets regularity and smoothness.
The method, which is applied by Autofit, is known under the name of active contour model or snakes. Kass, Witkin and Terzopoulos published the seminal work on the method.
M. Kass and A. P. Witkin and D. Terzopoulos (1988), Snakes: Active Contour Models, International Journal of Computer Vision, 1(4): 321-331

The Autofit algorithm in Imaris is based on the following publication:
Donna J. Williams and Mubarak Shah (1992), A Fast Algorithm for Active Contours and Curvature Estimation, CVGIP: Image. Understanding, 55, (1), 14-26.

Please Note: The Autofit algorithm does not solve the entire problem of finding contours in images, but rather, depends on mechanisms like interaction with a user, interaction with some higher level image understanding process and information from image data.The accuracy and computation time depends on the convergence criteria used in the Autofit.The model is sensitive to the initial contour line estimate and algorithm parameters. The automatically determined parameters provide the optimal values for the Autofit algorithm.

Perform Autofit button
Clicking on this button performs an Autofit calculation.

Presets

Shape Smooth slider Accurate
This setting affects the shape of the resulting contour line. If the Shape slider is set to be more on one side the resulting contour line can be encouraged either to be **smooth** or more **accurate** with original image intensity gradients.

Impact Weak slider Strong
The impact signifies how strongly the algorithm will affect the contour line.
If the impact is set to be **Weak**, the algorithm performs only a few iterations and does not move the vertex very far from the initial contour line.
If the impact is set to be **Strong**, the number of iterations will increase and the vertexes can be moved to find edges that are more distant from the initial contour line.

Advanced
This option activates only if the **Presets** option is not selected.

Continuity
The Continuity value affects the vertices space regularity and tends to keep the distances between each pair of adjacent vertices equal. Setting a high continuity value enforces the contour vertices to be equally spaced on the line.

Curvature
The Curvature value constrains the shape of the contour line. Setting a high value for Curvature, results in a circularly shaped smooth contour line.

Image Energy
The Image Energy value constrains the intensity gradients on the contour. Setting a high value places the contour line at the intensity high gradients.

Reach Distance um
This parameter controls the maximal distance the contour vertex can move away from the original position during a single iteration.
Setting a high value enables the algorithm to find edges at more distant locations from the initial contour line and therefore decreases the risk of falling into local minima. However, the algorithm then has a tendency of detecting edges different from the desired object.

Max iteration
This parameter defines the Maximum number of iterations to be performed. The Autofit algorithm terminates if the specified maximum iterations have been performed.

SSC iterations
Scale space continuation is a method for preventing the Autofit algorithm to place a contour line at the local minima created by noise. First a large Gauss filter is used and then the Autofit algorithm iterates while slowly

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reducing the size of the Gauss filter. SSC iterations define the number of Autofit algorithm iterations
For example, if the maximum number of iterations is set to 30 and the number of SSC iterations is set to 3, the Autofit algorithm is run three times. The first time with a Gauss filter with three times the specified standard deviation, then for the second time with two times the standard deviation and finally, for the third time with the specified standard deviation. 10 iterations are performed maximally each time.

Gauss numerical field um
The standard deviation of the Gauss filter applied prior to the algorithm.

Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

Slice (data field)

The respective slice number is displayed in the number field.
The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and than simply click at the drawing plane and drag it to a new position.

Lock button

This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.
Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.
The L key allows the switch between locked and unlocked orthogonal slicer.
Please refer to the chapter Shortcuts Overview in the Reference Manual for a detailed list of shortcuts for manual surface creation.

Draw button

Click on this button to alter **between** the **two** Drawing Modes: **Active** and **Inactive.**

Drawing Modes

In the contour line creations, there are two different drawing modes: **Active** and **Inactive**.
Active
The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines.
Inactive
In the inactive drawing mode, the existing contour lines can be modified or deleted.
You can select the respective mode by using the the **Draw button** or **Ctrl+Space key**.
Press the **Draw button** or **Ctrl+Space key** to alter Drawing mode between **Active** and **Inactive** mode.

The pointer's appearance depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

Create Surface button

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

14.13.4.6 Shortcuts Overview

You can select the drawing mode by pressing once the **Ctrl+space** key. The pointer appearance on the screen depends on the selected drawing mode. The symbol on the screen appears as an **arrow** in the **Inactive** drawing mode and as a **cross** in the **Active** drawing mode.

Contour Line Mouse & Keyboard Functions	
up or down arrow keys	Move up / down one slice
left/right arrow keys	Move up / down to next slice which contains contour lines
C key	Copy contour lines from current slice
X key	Cut contour lines on current slice
V key	Paste contour lines to current slice
Del key	Delete all contour lines on current slice
O key	Smooth all contour lines on current slice
A key	Perform Autofit Algorithm on current slice
+ key or **.**	Increase contour line width
- key or **,**	Decrease contour line width
Mouse wheel	Change circle radius or magic wand tolerance
	Drawing mode **Active**
Backspace key	Abort drawing of currently opened contour line
Enter key	Stop drawing currently opened contour line and leave it open
U key	Change to contour line Union mode Union is indicated by a "+" sign next to the mouse cross cursor
S key	Change to contour line Subtraction mode Subtraction is indicated by a "-" sign next to the mouse cross cursor
	Drawing mode **Inactive**
L key	Toggle lock/unlock orthogonal slicer
T key + Left-click on line (vertex)	Translate contour line or vertex
R key + Left-click on line (vertex)	Rotate contour line
S key + Left-click on line (vertex)	Scale contour line
D key + Left-click on line (vertex)	Delete contour line or vertex

Union mode
The result of Union mode is a single contour line whose area is the minimum superset of the overlapping input contour line areas.

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Subtraction mode
The result of subtraction mode is a single contour line whose area is the area of the original line minus the operator line area.

14.13.5 Tab Edit

Here you find the manual editing tools for the Surfaces.

Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.

Edit Mode

Cut
This activates the Surface Scissors function for manually spiting two touching surface objects. If you select **Cut** as edit mode, the Cut Properties are available.

Scissors Properties

Use the Surface Scissors to manually split two touching Surface objects.

Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.

- Change to the pointer mode Select.
- Select as Edit Mode the mode Cut.
- Shift + click on the Surface object.
- A colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular).
- Click on the button **Cut Surface** and the object splits into two individual Surface objects with two different colors.

Button **Cut Surface**
Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

Button **Clear Scissor**
Click on this button to clear the selection.

Close Borders
Check this box to add a surface along the cutting line and close the surface.

Mouse selects
Specify what object is select by a single mouse click.

Surface
Track

Process Selection

Button **Clear**
Click this button to clear the Surfaces selection.

Button **Duplicate**
Click this button and all selected Surfaces appear in a new group in the Surpass Tree (Surfaces Selection).

Button **Delete**

Select a Surface and click on this button to delete the Surfaces.

Button **Duplicate to all Timepoints**
Click this button and the selection is not only active for the current time point but for all time points in which the object appears.

Button **Unify**
Click this button and the selected Surfaces are unify into one Surface object.

Please note: Selecting the Unify button will not connect or bridge the two objects by adding any new pixels. The new, unified object may consist of two or more unconnected components.

Button **Split**
Click this button and the selected Surfaces are separated into two Surface objects.

	PC:	Mac:
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

Mask Properties

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask All...**
Button **Mask Selection ...**
Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

Surfaces

In Imaris it its possible to merge two or several Surfaces items into one Surfaces item.

Button **Merge ...**
This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

Window: Mask Channel

Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

Duplicate Channel before applying Mask
When this option is selected, the channel will be duplicated before applying Mask.

Mask Settings

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Random Color Map
Constant Inside/Outside
Define, if you want to use a random color map or if you want to set a constant inside/outside.

Set Voxels outside Surface to:
The default value is 0.000, no voxels outside the region of interest are displayed.

Set Voxels inside Surface to:
If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

Apply to all Time Points
Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button OK
If you want to apply the mask click on **OK**.

Button Cancel
If you do not want to apply the mask click on **Cancel**.

Window: Merge selected Objects to "Surfaces X"

Check the boxes in front of the desired Surfaces or use the button Select All.

Button Clear All
Use this button to clear the Surfaces selection.

Button OK
If you want to merge the Surfaces click on **OK**.

Button Cancel
If you do not want to merge the Surfaces click on **Cancel**.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

14.13.6 Tab Edit Tracks

Mouse Selects

This option allows you to select an object within an image according to its object type by a single mouse click. To edit the properties of multiple objects simultaneously, select the required object type.
There are two options:
Surface
Track

Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.
The first row of the track-editing table displays the timeline. Each number represents a time point in the series.
The second row of the track-editing table displays the **object name**.
Multiple object types can be tracked easily since every object type appears in different colors.

Object ID

If the mouse pointer is on the object, a tooltip appears that identifies the object ID. The ID numbers of the objects are sequentially ordered column-wise from the top to the bottom of the table. The object maintains its unique ID number independent of the track being allocated to.
If the mouse pointer is on the connection, a tooltip appears that identifies the track ID number.

Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.
In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.
For multiple selections press Ctrl+click (Mac: Command+click).

Visualization options

Zoom
Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.
Using the mouse wheel will have the same affect.
Pan
To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

Time indicator
The first row of the track-editing table displays the timeline. Each number represents a time point in the series. The current time point is indicated by vertical line highlighted with in current selection color.

To modify the time point, do one of the following:
1. Moving the slider in the Time bar modifies its position in time and changes the time point in the track editor.
2. Clicking on the time point within the track-editing table automatically moves the time line indicator to the corresponding point.
Double clicking on the time indicator, fixes the position of the current time point at the center of the track-editing table, regardless of the selected zoom or pan options)

Editing

Track Editor has several features that help you modify your tracks

Add tracks

To set the starting point of a new connection first you have to select the object.
Shift+left-click followed by a mouse movement on the object creates the new connection.
To establish a connection, drag the edge to the target object. If valid, the target object is highlighted (red).
Release the mouse button and the line segment clips automatically onto the new object, creating a new connection between the two objects.

Please note: The new connection is only possible between the objects of the same type. If you release the mouse button without being on a target object, the edge will disappear and a new connection will not be established.

A connection between objects of the same time point is also invalid.

Delete Tracks Edges

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Tracks
Select the track and press backspace to delete the whole track.

Connections
To delete the connection, select the connection and press shift-click. The selected connection is deleted.

The process of deleting the connection splits the track. The first track part remains with the same ID number and stops at the time point of the deletion. The second part consists of the remaining deleted objects and the connections between them. It is automatically shifted to the bottom of the track-editing table and assigned a new track ID number.

Connect unconnected components

Manually added, or previously untracked objects are displayed as separated circles. The procedure of creating new connections and connecting the objects is unchanged.

Show selection (build working set)

This option allows you to work on subset of tracks.

Connect Track Button

Click this button to connect two existing tracks. Select two Spots in two different tracks at the subsequent time points (just one Spot per time point) and click on the button Connect Track. The connection is automatically created between all selected objects.

Disconnect Track Button

Select a object and click on the button Disconnect Track. The connection from the selected object to the next object in time sequence is deleted.

Correct Drift

If you have an object drift (e.g. a cell drift) during the image acquisition you can use the Correct Drift button to correct the drift for the whole data set.

The ImarisTrack tracks all objects in an image, even the objects that should be stationary.
The Correct Drift function gives the user the opportunity:

1) to specify the tracks related to the stationary objects
2) to assign these objects as stationary objects and
3) to adjust the position of the rest of the image objects based on this selection.

As a result of drift correction, the entire image is transformed in such a way that the selected objects remain stationary and within the new image each objects' position (changes) based on the correct draft algorithm. Within the new image, the true movement of the objects can be then detected relative to the stationary objects.

In the accordance with the selected for **Result DataSets** options. the new image coordinates are calculated and displayed.

To make the Drift Correction on the data set the following steps are completed:

- Select the tracks associated with the stationary objects
- Select the algorithm for drift correction
- Select the option for the Result data sets
- Verify the selection and click on the OK button.

Please note: The drift correction changes the data set.

Based on the provided the track sub-selection the algorithm determines the new objects position for all time points.
If the tracks are selected, the algorithm will be performed on the sub selection. Otherwise, every track of the image is considered for calculations.

Algorithms

The Drift Correction is calculated based on the two algorithms, Translation and translation and rotation.

Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

Please note: The number of the selected tracks affects the algorithm calculation.

Selection of a single track gives a complete description of a translation.
Selection of a two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).
A better fit between the time points is achieved when three or more tracks are selected.

Result DataSet size

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.
Select the Result data set option to determine which new image part will be displayed.

Crop largest common region

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

New size equal to current size

The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

Include entire result

The final image will be the size of the combined area of the original and corrected image.
Selecting the appropriate Result dataset size may speed up the data visualization. This is especially important with (with large data sets.)

Correct spots positions

Activate this parameter and the spots are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

All actions in the Draw , Edit and Edit Tracks tabs can be Undone/Redone by selecting the Undo/Redo command or by pressing Ctrl+Z/Ctrl+Y.

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14.13.7 Tab Filter

Please note: Before using you have to add a filter to the list (see below: button Add)!

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.
The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

By using multiple filter types, more than one criterion can be specified for the data analyses.

Filters

Add Button
To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

Delete Button
Select a filter in the list with a left mouse click and click on this button to delete the filter.

Filter Type

Select in the drop down list the filter you want to apply.

Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the or button. The Red

button indicates that the threshold option is currently inactive, while the green button indicates an active threshold.
In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

Automatic/Manual A/M button
The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.
When the lower/upper threshold values are not activated (the red button) the corresponding A/M button is light gray indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button), the corresponding A/M button turns black.
After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form indicating an activated Automatic threshold to indicating a Manual threshold.
Clicking on the histogram automatically activates a Manual threshold adjustment.

Lower Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

Higher Threshold

Automatic
The initial threshold value is automatically computed.
Manual
Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.
To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold and to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.
The numerical range of the statistics value (e.g. Quality)and the number and percentage of objects that fall into the particular filtering class are displayed at the bottom of the threshold histogram.

Duplicate Selection to New Surfaces Button
Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

See also:
Surpass View - Overview - Surpass Tree

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14.13.8 Tab Statistics

Surfaces statistics are automatically computed. For the statistical values please refer to the chapter Menu Edit - Preferences... - Statistics - Surfaces. The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different datasets through summarized statistical values and graphical data presentations.
Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:
a) Overall
b) Detailed
c) Selection
In the statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.
To sort the columns click on the arrow in column heading. Click again to reverse the sort.

Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

Category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.
Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.
In Imaris there are following Filter Categories: Surface, Surface Tracks, Spots, Spots Tracks, Dendrites, Dendrites Tracks, Filament, Filament Tracks, Spines, Spine Tracks, Point, Point Tracks, Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle and Vesicles Tracks.
These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.
To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical values from the list (turns the category button into a light gray color).
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Detailed Tab

A pull down list offers three types of **Detailed** statistical calculations:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking

on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific Values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.
Object Statistical Annotation option is available for Specific values parameters.

Average values
The Average table contains values determined on the basis of all values within a set of the selected variable.
Columns are labeled as follows:
Min (Minimum)- the smallest value within a set of values of the selected variable
Max (Maximum)- the largest value within a set of values of the selected variable
Mean – value of the sum of all values divided by number of values within a set
Std Dev (Standard deviation)- a measure of the values distribution around the mean values
Median- the middle value of the sorted set of variables
Sum- the sum of all values within a variable set
Count- number of values within a set
Unit- variable unit
Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Statistical variable classification:

1. Statistical variables associated with objects (Cells, Nucleus, Vesicles -volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables the Average values are displayed per time point. The changing the time points, statistical variable values and images are consequently changed.
2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement length, track intensity) are not time dependent.

All values
If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.
Object Statistical Annotation option is available for Specific values parameters.

Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).
A pull down list offers three types of statistical calculations from a Selection:
Specific Values
Average Values
All Values

Time Plots
The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.
Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

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Interactive two-way Selection
A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

Specific values
In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.
Object Statistical Annotation option is available for Specific values parameters.

Average values
The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

All values
If the option All values is selected, all statistical values will be displayed for the selected image element.
Object Statistical Annotation option is available for Specific values parameters.

Buttons Overview

	PC:	Mac:
Select Surface	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click

Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.
Click on the button **Settings** and you switch to the window Preferences - Statistics - Surfaces.

Button **Search** - Search by object ID.
Type in the object ID in the data field. Click on the button **Search**.
- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Surfaces** - Duplicate Selection to new Surfaces.
Click on this button and all selected elements are copied to a new Surface. The new Surface appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation**
Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** or **All Values** are selected.

Button **Export** - Export Statistics on Tab Display to File.
Only the statistics that are shown within the current tab will be exported.

Button **Export** - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button Export - Export All Statistics to File.
All of the statistics (that are enabled in the statistics settings) will be exported.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:
Menu Edit - Preferences... - Statistics - Surfaces

14.13.8.1 Statistical Annotations

Statistical annotations provides the opportunity to view and annotate image objects with the statistical data values. Using this function, the individual objects in an image are labeled with the specified statistical values and the statistical data is displayed directly in the viewing area
Imaris objects can be labeled with a set of any number of the user-defined statistical values.
The Statistical Annotation button provides a convenient interface in order to label the object with the statistical data. Click this button and an object statistical annotation will be automatically generated in the viewing area. The selected object is annotated for all time points with the specified statistical values.

How to add Statistical Annotation

In the Statistical tab choose the **Detailed** or **Selection** Tab.
In the viewing area select the object or group of objects. Change to the pointer mode Select and click on an image object that has been detected/segmented. For multiple selections press Ctrl+click (Mac: Command-click)
In the **Detailed** or **Selection Tab** a pull down list offers **Specific Values** or **All values** as types of statistical parameters.
Select the statistical value associated with the particular object type you are working with.
Press the Statistical Annotation button .
In the viewing area the object label appears showing selected statistical values as a statistical annotation.

The statistical annotation shows by default the name of the selected statistical parameter and its value.
The color white displays the text of annotations associated with statistical values.

For a single object, all selected statistical parameters are placed in the same annotation box. Once the object is labeled, selecting the additional statistical parameter updates the object statistical annotation; the new parameter and its value is added in the viewing area.
If the image is a time series, the statistical annotation will be visible in the viewing area for all time points. Selecting a statistical variable that changes over time creates an statistical annotation that consequently changes with every time point.
The statistical annotation displays values always for the current time point.

The InPress options will allow you to modify and specify the contents and format of the object labels.
To modify and adjust the statistical label click on the Icon InPress , press Ctrl + Shift + P, or in the menu bar select File – InPress.

14.13.9 Tab Color

Tab Color edit, manage and assign colors material to the Surfaces or Tracks.

Object Type

Choice of Object type is presented in drop down window. Select either Surfaces or Tracks and depending on

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the chosen object type selection of the variables is listed.
For **Object Type Surfaces** two color types are available:
Base
Statistics Coded

For **Object Type Tracks** three color types are available:
Base
Statistics Coded
Time Mapped

14.13.9.1 Color Type-Base

Tab Color edit, manage and assign material colors of a Surfaces/Track.

RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

Light reflection

Quality of the object material is further characterized by reflection properties.
Diffusion- diffuses reflection of a matte, uneven material.
Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

Palette

Palette- this option provides a system of preset colors organized into color schemes.
In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, artdeco, autumn, glass, metal, neon, rococo, santafe, sheen, silky, spring, summer, tropical, winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

14.13.9.2 Color Type-Statistics Coded

Statistics Coded

Depending on the object type selection (either Surfaces or Tracks) the Statistical variable selection is listed in the drop down window. For example, if Surfaces is chosen as the Object type, available Statistical type will be the Surface linked variable. Similarly, Statistical variables for Tracks will be listed, if Object type is selected to be Tracks.

Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area. The upper one is the colorbar range for Surfaces and lower one is the colorbar for the Tracks.

Show Colorbar
Check the box to see the statistics colorbar in the viewing area.

Show Title
Check this box to see the title in the statistics colorbar.

Show Range
Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

Colormap

Button **Reset**
Click the **Reset** button to set the image back to the original values.

Button **Load**...
Click on the button **Load** to display the Import Color Table File color window. Enter the path and the requested File name and/or File type and click Open. The selected file is loaded and displayed.

Colormap range

Colormap range-specify the limits for colorbar.
Min - lower color limit
Max - upper color limit
Button **Auto**
Click the button **Auto** to detect the system the real min and max values and sets the Max. and Min. limits automatically to these values.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

14.13.9.3 Color Type-Time Mapped

Time Mapped
This option is only available when Tracks (see above) is selected as Object Type. The color range for the Tracks goes from blue to white to the range of time points.

Transparency

Transparency defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

14.14 Volume

How to Add a new Volume?

- Select menu Surpass - Volume.

The Surpass Tree list is automatically updated and in the Volume - Properties the Settings parameter are displayed. A data set can have only one volume.

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14.14.1 Display Adjustments

To set the correct Display Adjustments for the Volume rendering of your data set please refer to chapter Menu Eidit - Show Display Adjustment.

14.14.2 Tab Settings

Mode

MIP (max)
In the Maximum Intensity Projection mode only the brightest point (the point with maximum intensity) of all layers along the viewing direction displays. If your data set has more then one channel the combination color displays. A layer in the data set with high red and high green intensity displays yellow.

Normal Shading
The result of the normal shading is a depth effect on the screen. The surfaces turned away from the light source appear darker.

MIP (min)
A Maximum Intensity Projection with minimum intensity of all layers along the viewing direction.

Blend
The result of the bland mode is a depth effect on the screen. One effect of the blend mode is, that the borders appear darker. In the blend mode all values along the viewing direction including their transparency are used for the calculation.

Shadow Projection
The result of the shadow projection is a depth effect on the screen. An object between light source and the surface you look at projects a shadow.

If Shadow Projection is selected, the parameter Fix Light(s) to Data Set is available.

Fix Light(s) to Data Set
Check the box to fix the x-, y-, and z-axes to the same side when navigating the object. When the box is unchecked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

This check-box is only available, if Shadow Projection is selected.

Rendering Quality

If the rendering quality is set to 1.000, then 1pixel represents 1 voxel. If you decrease the rendering quality the image becomes coarse-grained.

View Aligned Planes
Check this box to see the display planes. Examine the planes while rotating the data set in the viewing area.

See also:
Preface - Terminology

14.14.3 Tab Draw

Volume Painter

On the tab Draw you can find the settings for the Volume Painter. Use this Volume Painter to adjust the voxel

intensities before you do a segmentation based on Surfaces, Spots, or Filament. (If an automatic algorithm is not able to separate two objects, the intensity between these two objects can be "erased" in advance.) You can draw disks, spheres, or hallow spheres into the selected image channel.

- First select the desired channel and painter shape.
- Rotate the view to execute a vertical cut.
- Then customize the painter settings.
- Change to the pointer mode Select.
- Move the cursor to the desired position.
- Hold down the Shift-key and click to add the shape.
- In the viewing area you see a preview of the painter tool.
- To re-edit a shape use the point selection.
- Set intensity to 0 to erase a region, or set the intensity to 255 to fill a region.
- To fix the shape click on the button **Set Intensity**.

Please note: The button Set Intensity changes the data set.

Selected Channel

Select the respective source channel.

Painter Shape

Here you select the painter shape.

Disk
The painter is disk shaped. In the settings selection adjust the diameter, width and intensity of the disk (see below).

Sphere
The painter is a sphere. In the settings selection adjust the diameter and intensity of the sphere (see below).

Hollow Sphere
The painter is a hollow sphere. In the settings selection adjust the diameter and intensity of the hollow sphere (see below).

Selection

Diameter
Here you select the diameter of a disk, sphere, and hollow sphere. If your mouse has a wheel, you can use the wheel to adjust the diameter.

Width
Here you select the width of a disk.

Intensity
Here you select the painter shape intensity. The shape preview in the viewing area changes accordingly. If you want to cut parts out of the channel select 0 as intensity value.

Button **Set Intensity**
In the last step you click on this button to fix the shapes. The button **Set Intensity** changes the data set and it is not possible to re-edit the shapes again.

Button **Delete All**
Click on this button to delete all inserted shapes in the preview.

Selected Point

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You can use the following data fields and the button **Delete** to re-edit or delete a selected point.

Position [um] X, Y, Z
To select a point change to the pointer mode Select and click on a point. The color of the selected point changes. The point position displays in the x, y, and z data field. To move the point click on the new position in the viewing area or edit the x, y, or z data field.

Button **Delete**
Click on this button to delete the selected point.

14.14.4 Tab Statistics

Volume statistics are automatically computed. For the statistical variable values please refer to the chapter Menu Edit - Preferences... - Statistics - Volume.Statistic tab collects and displays image statistical values in Imaris. Statistic tab enable us to understand and compare different datasets through summarized statistical values and graphical data presentations.
Several types of statistical tools enabling convenient organization and analysis of heterogeneous statistical data. Statistical View is configured as follows:
a) Overall
b) Detailed
c) Selection
In statistical dataset the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.
To sort columns click on the arrow in column heading. Click again to reverse the sort.

Overall Tab

Output of the **Overall** statistics provides an overview of the image single determining variables.

Buttons at the bottom of the Statistics tab

Button **Settings** - Configure List of visible statistics values.
Click on the button **Settings** and you switch to the window Preferences - Statistics - Volume.

Button **Search** - Search by object ID.
Type in the object ID in the data field. Click on the button **Search**.
- Highlight the object in viewing area
- Highlight the object on the Detailed Tab
- Display of the values on the Selection Tab

Button **Surfaces** - Duplicate Selection to new Surfaces.
Click on this button and all selected objects are copied to a new group. The new group displays in the Surpass Tree.

Button **Export** - Export Statistics on Tab Display to File.

Button **Export** - Export selected Data for plotting
Exports the selected statistical value for plotting. The selected value has to be time dependent.
With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.
With a click on the button **Export** you open the Window Export Statistics. Select the file type (Comma

Separated Values *.csv, Excel file *.xls, or Excel XML *.xlm). If you select MS Excel, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:
Menu Edit - Preferences... - Statistics - Volume

15 Animation

The Key Frame Animation function defines several subsequent views (key frames) of your image, which will be used to create an animation. Imaris generates smooth transitions between the selected views by interpolation.

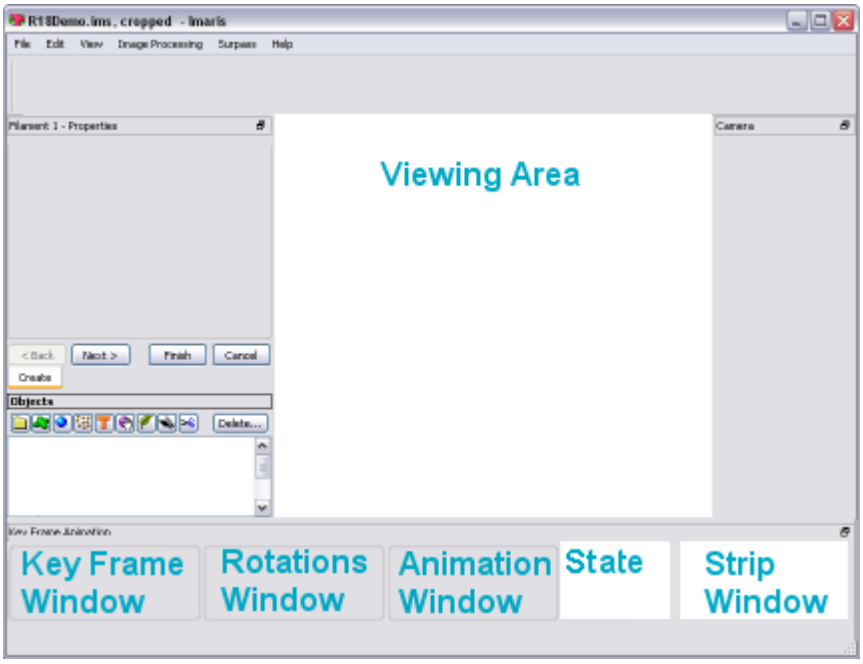
How to Open the Key Frame Animation Window?

- Click on the icon in the Main toolbar.
- Select menu View - Animation.
- Press the key combination Ctrl + 6.

See also:
Menu Edit - Preferences... - Surpass
Animation - Mouse & Keyboard Mac

15.1 Overview

The Key Frame Animation consists of different areas:



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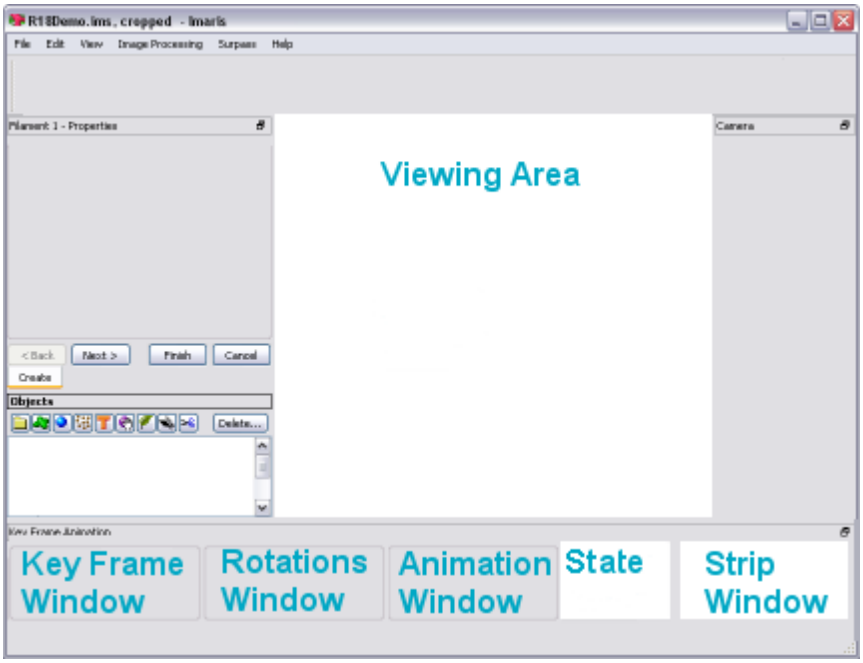

At the bottom of the screen the following windows are displayed: the Key Frame Window, the Rotations Window, the Animation Window, the State selection, and the Strip Window.

15.2 Key Frame Window

In the Key Frame window you define and manage the key frames of your movie.

Button **Add**
Use this button to insert user-defined key frames in the strip sequence.

Button **Modify**
Use this button to modify user-defined key frames in the strip sequence.

Button **Delete**
Use this button to delete user-defined key frames in the strip sequence.

Button **Delete All**
Click on this button to delete all user-defined key frames in the Strip window.

Large spaces between the user-defined key frames (thick blue lines) will cause that section of the movie to play more slowly. Placing the lines close together will cause that section of the movie to play more quickly. Use this button to set a constant speed throughout the entire movie.

Button **II<**
Button **II>**
With the arrow buttons you step to the previous (arrow to the left) or next (arrow to the right) user-defined key frame.

Buttons Overview

Add	**Add** new user-defined key frame
Mod.	**Modify** active, user-defined key frame

Del	**Delete** active, user-defined key frame
Del. All	**Delete** all key frames
II<	Go to **Previous** user-defined key frame
II>	Go to **Next** user-defined key frame

For the complex use of these buttons please refer to chapter Animation - Strip Window.

See also:
Animation - Create Animation

15.3 Rotations Window

Use the templates to quickly define standard camera motions for the key frame animation system.

Templates
Select one of the predefined templates. The key frames are inserted automatically. Click on the button **Play** to see the animation.

Button Custom
Click on this button to display the Custom Rotation window to freely define the rotation (see below).

Space

Screen
The rotation axis is associated to the x-, y-, and z-axis of the screen.

World
The rotation axis is associated to the x-, y-, and z-axis of the data set.

Axis

X
Y
Z
Turn along the x-, y-, or z-axis.

Direction

+
Turn to the left.

-
Turn to the right.

Angle

Select or type in the desired angle.

Button OK
Click on this button to accept the changes.

Button Cancel
Click on this button to cancel the changes.

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15.4 Animation Window

Frames
The number in the filed **Frames** indicates the number of thin blue lines within the Strip Panel. This number defines the duration of animations.

Button **Settings ...**
To open and adjust the Key Frame settings click on this button. For details please refer to chapter Menu Edit - Preferences... - Surpass.

Button >
Click on this button (arrow to the right - **Play/Pause**) to start the movie preview. To pause the movie preview re-click on this button.

Button O
To record the movie click on this button (red dot - **Record**). Selecting this button the Save As Movie window is displayed (see below).

Window: Save as Movie Window

File name
Enter a file name.

Save as type
Select the desired file type (QuickTimeMovie, TIFF series, or AVI).

Movie Settings

Compression Factor
Select a compression factor between 0 (high quality) and 100 (low quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

Frame Rate
Define how many frames are displayed per second.

QTVR Settings

The QuickTimeVR Settings are not available.

Add to ImageAccess database
Check the box to add the file to the ImageAccess database.

Play Movie with default Media Player when finished
Check the box to play the movie with the default media player when finished.

Menu Edit - Preferences... - Surpass (default Key Frame settings)
Surpass View - Overview - QuickTimeVR

15.5 Play Back State

In the State selection you can adjust the play back settings.

Camera
- Check: Play back the previously recorded Camera positions for each key frame.
- Uncheck: Do not play back the recorded Camera positions. The Camera stays in the same position. You

can change the Camera position manually while playing the animation.

Time Points
- Check: Play back the previously recorded Time Points for each key frame.
- Uncheck: Do not play back the recorded Time Points. The Time Points do not automatically change during the animation. You can change the Time Points manually while playing the animation.

Colors
- Check: Play back the previously recorded Colors for each key frame.
- Uncheck: Do not play back the recorded Colors. The Colors do not automatically change during the animation. You can change the Colors manually while playing the animation.

Display Adjustments
- Check: Play back the previously recorded Display Adjustments for each key frame.
- Un-check: Do not play back the recorded Display Adjustments. The Display Adjustments do not automatically change during the animation. You can change the Display Adjustments manually while playing the animation.

Clipping Plane (Position)
- Check: Play back the previously recorded Clipping Plane positions for each key frame.
- Uncheck: Do not play back the recorded Clipping Plane positions. The Clipping Plane positions do not automatically change during the animation. You can change the Clipping Plane positions manually while playing the animation.

Orthogonal Slicer (Position)
- Check: Play back the previously recorded Orthogonal Slicer positions for each key frame.
- Uncheck: Do not play back the recorded Orthogonal Slicer positions. The Orthogonal Slicer positions do not automatically change during the animation. You can change the Orthogonal Slicer positions manually while playing the animation.

Oblique Slicer (Position)
- Check: Play back the previously recorded Oblique Slicer positions for each key frame.
- Uncheck: Do not play back the recorded Oblique Slicer positions. The Oblique Slicer positions do not automatically change during the animation. You can change the Oblique Slicer positions manually while playing the animation.

Objects Show/Hide (Object Visibility)
- Check: Play back the previously recorded objects for each key frame.
- Uncheck: The visibility of the objects in the viewing area can be changed manually during the animation. Check or un-check Surpass Tree Items during the animation and the effect is directly visible in the viewing area.

See also:
Animation - Create Animation

15.6 Strip Window

The Strip window provides the working area. To display the views of the different key frames left-click on a line to display the corresponding image in the viewing area.

Lines in the Strip Window

Thick Blue Line
A thick blue line indicates a user-defined key frame.

White Line

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A white line represents the active key frame.

Thin Blue Line
A thin blue line stands for an interpolated frame.

Actions in the Strip Window

Add user-defined Key Frame
- Click on a thin blue line (interpolated key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add** to insert a user-defined key frame in the strip sequence.
- Click on a thick blue line (user-defined key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add**. Each time you click on the button Add you insert a new user-defined key frame right to the last active key frame.

Please remember: If you select a thin blue line, you define a new user-defined key frame exactly on this location. If you select an already user-defined key frame the new key frame is halfway between the selected and the next user-defined key frame on the right hand side.

Delete user-defined Key Frame
Click on a key frame and then on the button **Delete**.

Move user-defined Key Frame
To move a key frame in the Strip window drag the key frame line while holding down the mouse button. The first and last key frames can not be moved.

Modify user-defined Key Frame
Select the key frame in the Strip window. Turn the image to a new position and click on the button **Modify**. The new position for the selected key frame is saved.

Copy user-defined Key Frame
To copy a key frame in the Strip window select the initial key frame with a click. The scene in the viewing area changes accordingly. Right-click on the key frame in the Strip window at the desired copy position. The scene in the viewing area does not change. Click on the button **Add** to insert the user-defined key frame.

Please remember: Click on a key frame to change the camera position in the viewing area accordingly. Right-click (if you use a three button mouse) on a key frame and the view does not change.

Strip Window Overview

Click on key frame	Select key frame and change view accordingly
Right-click on key frame	Select key frame, view does not change
Button **Add**	**Add** new user-defined key frame
Button **Mod.**	**Modify** selected, user-defined key frame
Button **Del**	**Delete** selected, user-defined key frame

Menu Edit - Preferences... - Surpass (default interpolated frame settings)

15.7 Create Animation

The Key Frame Animation feature in Surpass allows you to create an animation from views (key frames). It interpolates frames between the user-defined views to create a smooth movie.

1. Adjust Pre-Settings

- Create an object (Surfaces, Volume, etc) from which to create the animation.

- In the Main toolbar click on the icon **Animation**. The Key Frame Animation window displays on the bottom of the screen and is divided in the Key Frame window, Rotations window, Animation window, State window, and Strip window.
- In the Animation window you find the data field Frames. Enter the number of frames to be included in the movie.
- Click the **Settings** button and enter the number of frames per second.

2. Add Key Frames

- Turn the image view to the first position.
- Capture the first view with a click on the button **Add** in the Key Frame window (records position). The key frame appears in the Strip window.
- Move the image to the next viewing position.
- Click on the button **Add** in the Key Frame window to set the next key frame position (records new position). The new key frame appears in the Strip window.
- Repeat as desired, until all user-defined key frames are added.

3. Select Play Back Settings

- In the State selection you can adjust the play back settings. Please refer to chapter Animation - Play Back State for details.

4. Play the Animation

- In the Animation window click on the button **Play** to start the animation.

Example: Handle Camera

Use 360° Template

Use the templates to quickly define standard camera motions for the key frame animation system.

- In the Rotations window select +360° Horizontal. Five key frames are inserted automatically. The first and the last key frames are identical.
- In the State window check Camera.
- In the Animation window click on the button **Play** to see the animation. The camera rotates 360° horizontally.

Capture Individual Camera Positions

- With the pointer in Navigate mode, position the image to create the view for the first key frame.
- In the Key Frame window click the button **Add**. With the first click on **Add** you insert the first and last key frames, they are identical. The key frames appears in the Strip window.
- Position the image to the desired view for the next key frame and click the **Add** button to place the next key frame.
- Repeat as desired, until all user-defined key frames are added.
- In the State window check Camera.
- To preview the movie, click in the Animation window on the button **Play**. The camera position changes automatically.

Move the Camera Position Manually During the Play Back

- Create an animation.
- In the State window uncheck Camera.
- Play the animation and change the Camera position during the play back manually.

Example: Handle the Orthogonal Slicer

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Individual Orthogonal Slicer Positions

- Add the Surpass Tree Item Orthogonal Slicer and check the box in the Surpass Tree.
- Adjust the Orthogonal Slicer for the first key frame and click on the button **Add**.
- Move the Orthogonal Slicer before you add the next key frame and so on.
- In the State window check Orthogonal Slicer.
- Play the animation. The Orthogonal Slicer position changes automatically.

Change Orthogonal Slicer Position Manually During the Play Back

- Create an animation.
- In the State window uncheck Orthogonal Slicer.
- Play the animation and change the Orthogonal Slicer position during the play back manually.

See also:
Animation - Overview
Animation - Key Frame Window
Animation - Rotations Window
Animation - Animation Window
Animation - Play Back State
Animation - Strip Window
Animation - Save Animation

15.8 Save Animation

When you are satisfied with the movie, there are two possibilities to save the animation; either as an Imaris Scene File or as a movie-file.
In Imaris as Scene File
Whenever you save the Scene File in Imaris, the actual animation is saved automatically as part of the Scene File. Open the menu File and select Export Scene and click on **Save**.

Save Different Animations in Different Scene Files
Saving different animations of the same data set requires saving of different Scene Files. After generating a new movie by the Key Frame Animation, rename each time the Scene File, e.g. add "Movie" at the end of the Scene File name. Open the menu File and select Export Scene... . In the dialog window type in the new Scene File name (e.g. PyramidalCellSceneAMovie.imx) and click on **Save**.

Record the Animation as Movie

To save the animation as movie click in the Animation window on the button **Record** (red dot). The Save As Movie dialog window is displayed on screen. As File name type in the corresponding Scene File name, select the file type and choose the Movie Settings. Click on **Save**.

The **Save as type** option allows you to select the movie output file format and save the settings as a preset for future use.

Imaris lets you **save** and **create movie** files on **any platform**. The movie file type specifies the video format and predetermines the codes required to view saved animation.
To playback the movie you must use the appropriate Media player for the selected movie file type.

Available Movie File Formats are:

MPEG-1 Movie (*.mpg)
MPEG-4 Movie (*.mp4)
AVI Movie (*.avi)

WMV Movie (*.wmv)
Quicktime Movie (*.mov)
Flash Movie (*.flv)
Raw Movie (*.avi)
Tiff (series) (*.tif, *.tiff)
Quicktime Virtual Reality Movie (*.mov)

Please note: The number and type of media player codes available depends on your operating system version, video card, and other software installed on your system.

If you have experienced problems with viewing the created animation files we recommend installing the free and platform independent, VLC player.

See also:
Menu File - Export Scene...
Surpass View - Overview - Scene File Concept
Surpass View - Overview - Quick TimeVR
Animation - Animation Window (Button Record)

15.9 Mouse & Keyboard PC

Animation Mouse & Keyboard Functions

Ctrl + 6	Activates the Key Frame Animation

In the Strip Window

Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

15.10 Mouse & Keyboard Mac

Animation Mouse & Keyboard Functions

Command + 6	Activates the Key Frame Animation

In the Strip Window

With a one-button mouse:

Click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Ctrl + click on key frame	Select key frame, view does not change

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

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Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

16 InMotion

The function InMotion is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression by a smooth animation of the view. This helps to get an understanding of the image depth, while manipulating with Surpass objects: Spots, Filaments, Measurement Points.

How to Start the InMotion Mode?

- Click on the icon in the Main toolbar.
- Select menu View - InMotion.
- Press the key combination Ctrl + 8.

To switch the mode off click on the icon Surpass in the Main toolbar.

Adjust 3D Cursor Settings

To adjust the 3D cursor settings click on menu Edit - Preferences... - 3D Cursor.

See also:
Toolbars - Main Toolbar
InMotion - Mouse & Keyboard Mac

16.1 Mouse & Keyboard PC

InMotion Mouse & Keyboard Functions

Ctrl + 8	Activates the InMotion function

16.2 Mouse & Keyboard Mac

InMotion Mouse & Keyboard Functions

Command + 8	Activates the InMotion function

17 Coloc

In fluorescent microscopy, colocalization describes the presence of two fluorochromes at the same physical location. ImarisColoc provides extended functionality for the analysis and visualization of colocalization in multi-channel data sets. It provides an automated selection of colocalization based on a method introduced by Costes & Lockett at the NIH, NCI/SAIC.

Coloc processes 2D, 3D, and 4D data sets. It operates on two channels simultaneously and measures the degree of overlap of the two channels.

With Coloc you can determine and display locations within the data set with common information from all

channels, e.g., to illustrate where all types of proteins can be found simultaneously or to detect low-density tissues in all channels.

The desired range for signal analysis can be limited by defining intensity thresholds or sets of intensity values for each of the involved channels. All voxels falling inside every of these channel-specific intensity limits represent a colocalization. They are emphasized in the view and statistics are calculated and displayed. When the selection is changed, the numeric and visual feedback happens in real-time.

The result of the colocalization as a whole can be saved as a separate channel, which can then be processed and viewed as any originally acquired channel in Imaris. Multiple colocalization images are easily combined into a single view in ImarisSurpass.

Coloc is an optional module within the Imaris program and is not available as a standalone program.

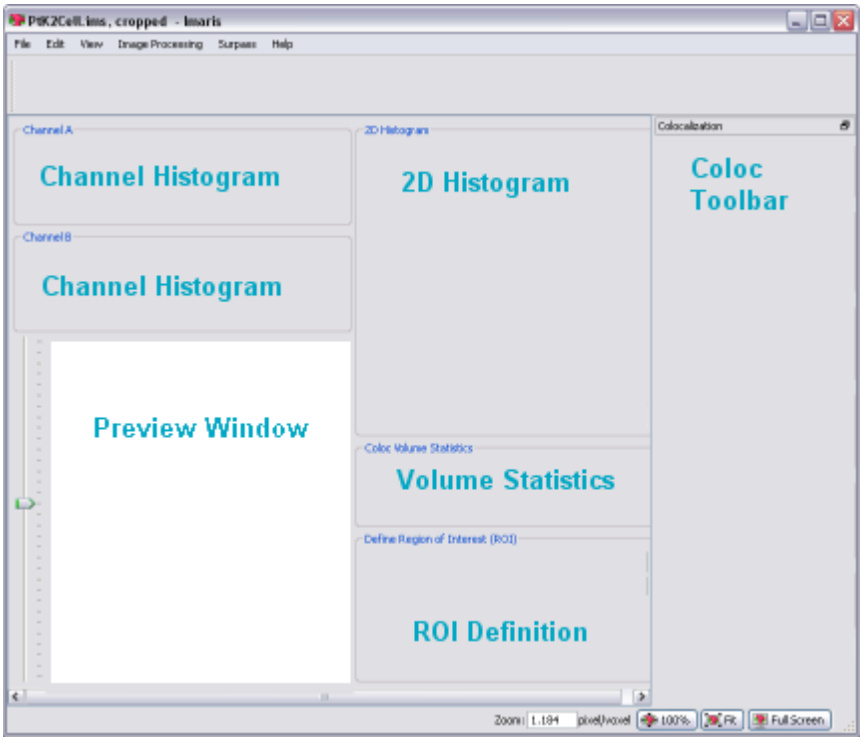
How to Display the Coloc Main Screen?

- Click on the icon in the Main toolbar.
- Select menu View - Coloc.
- Press the key combination Ctrl + 7.

See also:
Coloc - Mouse & Keyboard Mac

17.1 Overview

The Coloc main screen consists of different areas:



- Channel Histogram (channel A, channel B). This area allows the selection of the two channels for the colocalization analysis.
- Preview Window. This area visualizes the data set together with a preview of the colocalized region.
- 2D Histogram. This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity

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pairs considered as colocalized can be defined on the histogram.

- Volume Statistics. This area displays statistics about the resulting colocalized volume. The display adapts instantly to all modifications in the selection.
- ROI Definition. This feature allows you to define a region of interest (ROI) for the entire analysis.
- Coloc Toolbar. The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.

17.2 Channel Histogram

This area allows the selection of the two channels for the colocalization analysis. The intensity histograms are displayed for the currently selected channels.

Select Channel A and Channel B

The two channels for colocalization detection must be selected first.

- Select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

Adjust Threshold

- The threshold for each channel can then be defined by clicking in the histogram, dragging the colored line directly, or by entering the value in the threshold field.

Adjust Histogram Settings

If necessary, you can adjust in the Coloc toolbar the histogram mode and histogram options.

See also:
Coloc - Coloc Toolbar
Coloc - Mouse & Keyboard PC
Coloc - Mouse & Keyboard Mac

17.3 Preview Window

This area visualizes the data set together with a preview of the colocalized region. It displays a Slice representation of the data set, i.e. a 2D view from the top in the z-axis direction. On the left hand side, a sliding bar with a handle allows inspecting and displaying the single slices in the data set along the z-axis. The Preview window updates in real-time the display of the colocalized region based on the actual settings of the colocalization parameters. The original channels can be switched on or off in the Display Adjustments window.

Time Settings

There are no time-dependent parameters for the colocalization analysis. The Preview window always displays the currently selected time point of the data set. The values in the Statistics window always refer to the volume of the current time point. However, when the **Build Coloc Channel** button is pressed, the Coloc channel and statistical values for all time points are calculated.

See also:
Menu Edit - Show Display Adjustment
Coloc - Mouse & Keyboard PC
Coloc - Mouse & Keyboard Mac

17.4 2D Histogram

This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity pairs considered as colocalized can be defined on the histogram.

Adjust Channel Thresholds

Effects of the modification are instantly visible in the channel histogram A and B, and in the Preview window.

Selection Mode Threshold
The channel thresholds are visible in form of a rectangle.

- Point with the cursor on the 2D histogram.
- Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the edge and drag it to any another position in the histogram.

Selection Mode Polygon
The channel thresholds are visible as an intensity area in form of a free configurable polygon.

You draw the polygon on the 2D histogram. This method is especially useful for eliminating channel bleed through regions along the border of the lower intensity bins. After checking the Polygon mode, the overlaid threshold rectangle in the 2D histogram is converted into a polygon with four corner points. You can now start to modify this polygon or to draw a completely new one. The initial point of the polygon is visible as white filled.

Set Point
Press and hold the Shift-key. The cursor turns into a cross with a little sign. Click on the 2D histogram with the left mouse button to place a point (vertex). Any additional point connects with lines to the initial point and the last point inserted.

Insert Point in a Line
Press and hold the Shift-key. The cursor turns into a cross with a little " + " sign. Point on the line between two vertices and click on the line with the left mouse button to add a point.

Automatic Point Setting
Press and hold the Ctrl-key. The cursor turns into a cross with a little " + " sign and a stack symbol. Drag the cursor around. New vertices are added automatically at each change of cursor position.

Move Point
Point on one of the vertices, click & drag the vertex around to the desired location. The polygon changes its shape.

Move Line
Point on a line between two vertices, click & drag the line around to the desired location. The polygon changes its shape.

Move Polygon
Point somewhere beside of the polygon edge, click & drag the polygon around to the desired location.

Delete Point
Shift + click on a point to delete the point.

See also:
Coloc - Coloc Toolbar (selection Mode Threshold or Polygon)

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17.5 Volume Statistics

The values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. The values are calculated by the following formulas.

Please note: The calculated Coloc Volume Statistics are not automatically saved in the data set. To save or export the statistics you have to build a coloc channel (Coloc - Coloc Toolbar - Build Coloc Channel).

For publishing Coloc statistical values please use only the statistics reported in the Channel Properties.

Due to a real-time response, some of the statistical values shown in the main Coloc window are generated from a sub-sampled dataset. The accuracy of these values is depending on the variability introduced by sub-sampling procedure.
Depending on the dataset, the difference between the values reported in the Channel Properties (a high accuracy values, produced from the entire dataset) and these values (calculated from sub-sampled dataset) may differ significantly.

Definitions

Regions:

Data set	All data set voxels
Coloc	Colocalized voxels
Object A	Voxels with channel A intensity above threshold A
Object B	Voxels with channel B intensity above threshold B
ROI	Region of interest

Indicators:

N	Number of voxels
SAi	Channel A intensity of voxel
SBi	Channel B intensity of voxel
SAavg, Coloc	Average channel A intensity in the Coloc region
Volume A	Number of voxel channel A x voxel volume
Volume B	Number of voxel channel B x voxel volume
Material A	Channel A voxel signal intensity
Material B	Channel B voxel signal intensity

Number of colocalized voxels
Total count of colocalized voxels N_{coloc}.

% of data set colocalized
Percentage of total data set voxels colocalized.

$$\frac{N_{coloc}}{N_{dataset}} \cdot 100\%$$

% of volume A above threshold colocalized
Percentage of channel A voxels above threshold A colocalized.

$$\frac{N_{coloc}}{N_{objectA}} \cdot 100\%$$

% of volume B above threshold colocalized
Percentage of channel B voxels above threshold B colocalized.

$$\frac{N_{coloc}}{N_{objectB}} \cdot 100\%$$

% of material A above threshold colocalized
Percentage of channel A material above threshold A that is colocalized.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in objectA} SA_i} \cdot 100\%$$

% of material B above threshold colocalized
Percentage of channel B material above threshold B that is colocalized.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in objectB} SB_i} \cdot 100\%$$

% of ROI colocalized
Percentage colocalization of channel A and channel B volume inside the region of interest.

$$\frac{N_{coloc}}{N_{mask}} \cdot 100\%$$

% of ROI material A colocalized
Percentage colocalization of ROI channel A material.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in mask} SA_i} \cdot 100\%$$

% of ROI material B colocalized
Percentage colocalization of ROI channel B material.

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$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in mask} SB_i} \cdot 100\%$$

Pearson`s coefficient in colocalized volume
Pearson correlation of channel A and channel B inside the colocalized region.

Please note: The correlation value ranges between 1 and -1. A value of 1 represents perfect correlation, 0 no correlation, and -1 perfect inverse correlation.

$$R_{AB,coloc} = \frac{\sum_{i \in coloc} \left(SA_i - SA_{avg,coloc}\right)\left(SB_i - SB_{avg,coloc}\right)}{\sqrt{\sum_{i \in coloc} \left(SA_i - SA_{avg,coloc}\right)^2 \sum_{i \in coloc} \left(SB_i - SB_{avg,coloc}\right)^2}}$$

Pearson`s coefficient in data set volume
Pearson correlation of channel A and channel B inside the entire data set volume.

$$R_{AB,dataset} = \frac{\sum_{i \in dataset} \left(SA_i - SA_{avg,dataset}\right)\left(SB_i - SB_{avg,dataset}\right)}{\sqrt{\sum_{i \in dataset} \left(SA_i - SA_{avg,dataset}\right)^2 \sum_{i \in coloc} \left(SB_i - SB_{avg,dataset}\right)^2}}$$

Pearson`s coefficient in ROI volume
Pearson correlation of channel A and channel B inside the region of interest.

$$R_{AB,mask} = \frac{\sum_{i \in mask} \left(SA_i - SA_{avg,mask}\right)\left(SB_i - SB_{avg,mask}\right)}{\sqrt{\sum_{i \in mask} \left(SA_i - SA_{avg,mask}\right)^2 \sum_{i \in mask} \left(SB_i - SB_{avg,mask}\right)^2}}$$

Original Mander`s coefficient A
Original Mander's coefficient A = Sum(SAiSBcoloc) / Sum(SAi)
SBcoloc=1 if SBi>0, otherwise SBcoloc=0

Original Mander`s coefficient B

Original Mander's coefficient B = Sum(SBiSAcoloc) / Sum(SBi)
SAcoloc=1 if SAi>0, otherwise SAcoloc=0

Mander`s coefficient A (thresholded)
Thresholded Mander's coefficient A = Sum(SAiSBcoloc) / Sum(SAi)
SBcoloc=1 if SBi>thresholdB, otherwise SBcoloc=0

Mander`s coefficient B (thresholded)
Thresholded Mander's coefficient B = Sum(SBiSAcoloc) / Sum(SBi)
SAcoloc=1 if SAi>thresholdA, otherwise SAcoloc=0

See also:
Menu Edit - Image Properties... - Channel 1...n - Tab Coloc Statistics (to export the statistics)
Coloc - Coloc Toolbar - Build Coloc Channel (to build the coloc channel)

17.6 ROI Definition

Define Region of Interest (ROI)

This feature allows you to define a region of interest (ROI) for the entire analysis. A third channel can be selected as a masking area for the entire analysis. A masking channel is e.g. a third microscope channel acquired with settings that allows defining a region of interest by simple intensity thresholding. All voxels outside of the region of interest defined by the mask channel are ignored for the colocalization analysis. The volume excluded by the mask appears hatched in the Preview window. The mask channel is used in conjunction with the Automatic Threshold function. For this function, the selection of an appropriate region of interest is essential.

Mask Dataset
Check this box to activate the function.

X% of Data selected
Displays the percentage of selected data.

Channel Selection
Select the channel to be used from the drop-down list.

Channel Histogram
Point with the cursor into the histogram and click to set the threshold. A bolded line marks the threshold in the histogram and the corresponding value is indicated in the threshold field. The masked volume of the data set appears hatched in the Preview window. Drag the cursor in the histogram to modify the threshold. Effects of the modification are instantly visible in the threshold field as well as in the Preview window.

Threshold Value
Editable threshold value field.

Mask Channel and Automatic Threshold

When the mask is set as required, click the button **Automatic Thresholding** to calculate the thresholds. Build the colocalization channel by clicking the button **Build Coloc Channel**.

See also:
Coloc - Coloc Toolbar - Automatic Thresholding
Coloc - Coloc Toolbar - Build Coloc Channel
Coloc - Mouse & Keyboard PC
Coloc - Mouse & Keyboard Mac

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17.7 Coloc Toolbar

The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.

Selection Mode

You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bolded line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bolded lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bolded lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

Threshold
To adjust the threshold value using the mouse (see above 1. and 3.), select this mode.

Polygon
To draw a polygon line in the 2D histogram (see above 4.) select this selection mode.

Automatic Thresholding

Please refer to chapter Coloc - Coloc Toolbar - Automatic Thresholding.

Histogram Mode

The histogram mode options define the voxels selection for the colocalization calculation within the data set.

Single Slice
The 2D histogram is calculated and displayed for the current slice only.

Single Time Point
The 2D histogram is calculated and shown for the entire 3D stack of the current time point. This mode allows for faster browsing through the data set along the z-axis.

All Time Points
The 2D histogram is calculated and shown for an entire time data set, i.e. for all time points within the 4D data set.

Histogram Options

The histogram mode options are used to define the display of the 1D and 2D histograms.

Ignore Border Bins
A border bin is always the first and last value in a histogram. Often a lot of voxels fall into the bin with the lowest intensity. In a frequency plot this causes a massive and unnecessary downscale of all other intensity bins which are of more interest. The border bins can therefore be ignored when scaling the display of the histogram. Ignore is selected as default. With most data sets, checking the Ignore Border Bin and Logarithmic options allows a more representative histogram display.

Logarithmic
The frequency plot is shown in a logarithmic scaling instead of a linear scaling.

Color Coded
Applies a false color look-up table to the histogram to improve the visibility of intensity differences. The high frequencies (bins in the 2D histogram into which a lot of voxels accumulate) are shown in yellow-to-white, bins with little voxels are shown in blue-to-black.

Coloc Intensities

The Coloc intensities option defines the brightness of the calculated colocalization displayed in the Preview window.

Source Channels
Uses the intensities of the two channels for displaying the intensity of a colocalized voxel as the square root of the product of the intensities of the original channels.

Constant Value
Displays the intensity of a colocalized voxel defined by the specified value. No distinction is made whether high-intensity or low-intensity voxels colocalize as long as they are within the selection.

Coloc Color

Coloc color allows you to specify personal color settings for the colocalization display in the Preview window.

Button **Edit**
Clicking on the button **Edit** opens the Color window. You can either select one of the predefined colors to apply on the colocalization areas or define, add, and apply your own color definitions.

Button **Build Coloc Channel**
Please refer to chapter Coloc - Coloc Toolbar - Build Coloc Channel.

Button **Build Time Dep. Coloc**
Please refer to chapter Coloc - Coloc Toolbar - Build Coloc Channel.

Button **Channel Statistics**
Please refer to chapter Menu Edit - Image Properties... - Channel 1...n - Tab Coloc Statistics.

See also:
Coloc - Channel Histogram
Coloc - Preview Window
Coloc - 2D Histogram

17.7.1 Automatic Thresholding

Button **Calculate Thresholds**
This button performs an automatic threshold run on both channels based on an algorithm developed by Costes and Lockett at the National Institute of Health, NCI/SAIC, which is based on the exclusion of intensity pairs that exhibit no correlation (Pearson's correlation below zero).

The automatic threshold search is done using the 2D histogram. Starting with the highest intensity value, the algorithm reduces the threshold value step by step along a line (see figure below) and computes the correlation coefficient of the image using only voxels with intensities below the threshold. The algorithm continues reducing the thresholds until the correlation reaches 0, thus defining the automatic threshold.

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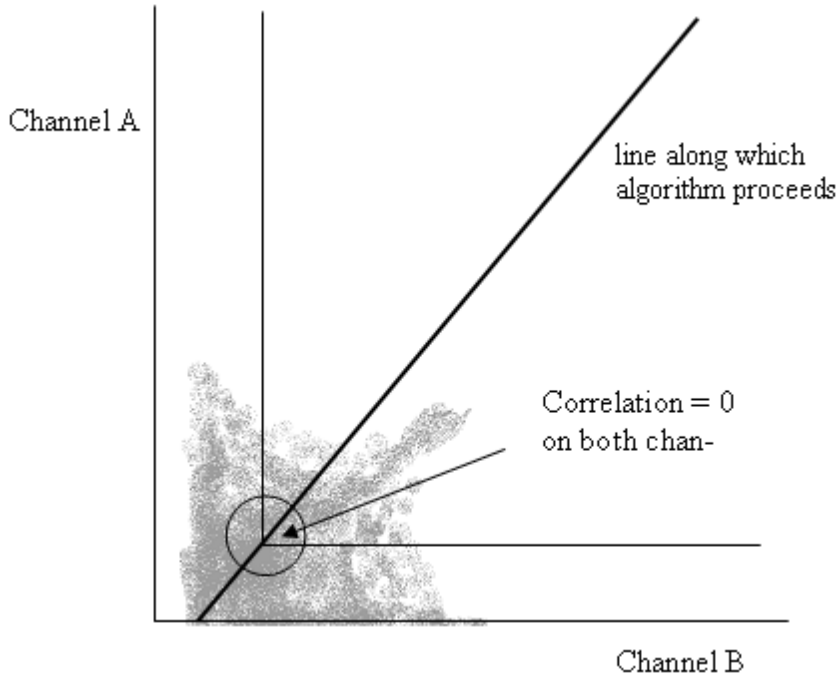

P-Value
PSF Width
As the size of the Point Spread Function please enter the length along the Z Axis. This can be a calculated theoretical length.
Links to calculators on the WWW you can be find e.g. on the Bitplane homepage.
The default in Imaris is not actually related to the PSF but only to the diagonal of the voxel size.

For more information about the P-Value and PSF Width please refer to the following publication:

Automatic and Quantitative Measurement of Protein-Protein Colocalization in Live Cells

Sylvain V. Costes,*y Dirk Daelemans,z Edward H. Cho,* Zachary Dobbin,* George Pavlakis,z
and Stephen Lockett*

* Image Analysis Laboratory, National Cancer Institute, Frederick, Maryland;
y National Cancer Institute/Science Applications International Corporation, Frederick, Maryland; and
z Human Retrovirus Section, National Cancer Institute, Frederick, Maryland

Biophysical Journal Volume 86 June 2004 3993-4003

17.7.2 Build Coloc Channel

Button **Build Coloc Channel**
Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set. The channel can then be saved and is part of the data set.

Button **Build Time Dep. Coloc**
Clicking this button calculates the colocalization channel according to the selected settings (calculating an automatic threshold individually for each time point) and adds it to the data set. The channel can then be saved and is part of the data set.

Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File – Save As or click the **Save as** button in the toolbar. The Save As box is displayed. Select the directory and enter the name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

Button **Channel Statistics** (for the new Coloc channel)
After building the Coloc channel, its statistics become available in the Image Properties. The button **Channel Statistics** displays directly the Image Properties window with the statistics for the calculated channel. The name of the channel, the description of the source channels and the selection method are indicated and displayed in the Image Properties on the Tab Coloc Statistics.

Export Statistics

The statistics can be exported.

Button **Export**
In the Image Properties window click the **Export** button. The Export Coloc Statistics window is displayed. Select the directory and enter the name for the file to be saved. The statistics are saved as .csv Excel file and can be opened directly with Microsoft Excel.

See also:
Coloc - Volume Statistics

17.8 Basic Operation

The basic use of Coloc in the daily work flow comprises the following steps:

- Select the channels.
- Adjust selection mode and histogram mode.
- Set thresholds.
- Check the statistics.
- Build and save the Coloc channel.

Select Channel A and Channel B

In the 1D histograms select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

Adjust Selection Mode and Histogram Mode

Before you set the thresholds, please check the appropriate parameters in the Coloc toolbar on the right hand side of the screen:

- Check as Selection Mode Threshold.
- Decide, which Histogram Mode you need. Choose Single Slice for calculating the histogram for the current slice only. Or choose Single Time Point to calculate the histogram for the entire 3D time point. Or choose All Time Points for the entire 4D image.

Set Thresholds

Colocalization occurs where the voxels are above threshold in channel A and channel B. You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bolded line on the channel histograms.

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2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bolded lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bolded lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

A. On the 1D Channel Histogram

- Point with the cursor on the histogram. The cursor turns into a cross.
- Click on the histogram to determine a threshold. The bolded left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the line and drag it to another position in the histogram. Effects of the modification are instantly visible in the other indications as well as in the Preview window.
- Perform for both channels until the settings are as desired.

B. By Entering a Value

- Click in the Threshold field of one of the channels and enter a value, then press Enter. The bolded left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot. Effects of the modification are instantly visible in the other indications as well as in the Preview display.
- Perform for both channels until the settings are as desired.

C. On the 2D Histogram

- Point with the cursor on the 2D histogram. The cursor turns into a cross.
- Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the edge and drag it to any another position in the histogram.

D. On the Preview Window

- Click with the cursor on the image in the Preview window. An active contour is displayed, outlining image elements brighter than the location clicked.
- Drag the line on the image to define the threshold of channel A. The selection works best when starting with brighter spots and dragging towards darker areas.
- Press and hold the Shift-key, click with the cursor on the image, then drag the line to determine the threshold of channel B.

Check the Statistics

The statistical values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. For details please refer to chapter Coloc - Volume Statistics.

Build Coloc Channel

Button **Build Coloc Channel**
Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set.

Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File - Save As or click the **Save as** button in the toolbar. The Save As box is displayed. Select the directory and enter the name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

17.8.1 Advanced Examples

The colocalization channel can be visualized like any other channel in Imaris. In the following examples, a Coloc channel was built on the retina data set.

Visualize the Coloc Channel in Easy 3D

Example 1: Visualize and Adjust the Coloc Channel in Imaris Easy 3D

- After building the Coloc channel, click on the **Easy 3D** button in the Imaris menu bar.
- Check the desired Rendering mode parameters and adjust the Settings if necessary, then click the **Calculate** button. The 3D image of channel is calculated and displayed.

As the colocalized region always lays inside the initial channels, making the initial channels invisible or more transparent allows a better view of the colocalized region.

- In the Imaris menu bar select Edit – Show Display Adjustment.
- In the Display Adjustments window uncheck one of the two original channels, then click the button **Calculate** again. The 3D image of the original and the Coloc channel is calculated and displayed.
- In the Imaris menu bar select Edit – Show Display Adjustment.
- In the Display Adjustment window select the Coloc channel and adjust its settings.
- In the Display Adjustment window select the remaining original channel and adjust its settings. In this example the channel is set as partly opaque.
- After each modification, click the **Calculate** button again to calculate the result.

Visualize the Coloc Channel in Surpass

The colocalization channel can be visualized like any other channel in Surpass.

Example 2: Create Surfaces of the Coloc Channel and Add the Two Initial Channels as Volume Rendering

- After building the Coloc channel, click on the **Surpass** button in the Imaris menu bar.
- In the Objects toolbar click the **Add New Surfaces** button.
- In the Surfaces Properties window check and select the Coloc channel.
- Select the desired parameters. In this example, we set the threshold just below the lowest intensity values in the histogram.
- In the menu Surpass select Volume.
- In the Imaris menu bar select Edit – Show Display Adjustment and uncheck the Coloc channel. The Surpass Volume object displays only the two initial channels. Then select the two initial channels and adjust their settings, e.g. decrease the blend opacity.

17.9 Mouse & Keyboard PC

Coloc Mouse & Keyboard Functions

Ctrl + 7	Activates the Coloc view

Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

Histogram Channel A and Histogram Channel B

Left-click on histogram	Set new threshold

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Left-click on line & drag	Move threshold

2D Histogram

Left-click	Set channel A and channel B thresholds
Left-click on line & drag	Move channel A and channel B thresholds

Preview Window

Left-click & drag	Set threshold channel A
Shift + left-click & drag	Set threshold channel B
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Selection Mode Polygon

2D Histogram

Shift + left-click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Ctrl + left-click	Add multiple new points
Left-click & drag (on point or line)	Move point or line
Left-click & drag (outside polygon)	Move polygon
Delete	Delete single point

17.10 Mouse & Keyboard Mac

Coloc Mouse & Keyboard Functions

Command + 7	Activates the Coloc view

Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

Histogram Channel A and Histogram Channel B

Click on histogram	Set new threshold
Click on line & drag	Move threshold

2D Histogram

Click	Set channel A and channel B thresholds
Click on line & drag	Move channel A and channel B thresholds

Preview Window

Click & drag	Set threshold channel A
Shift + click & drag	Set threshold channel B

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:
- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Selection Mode Polygon

2D Histogram

Shift + click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Command + click	Add multiple new points
Click & drag (on point or line)	Move point or line
Click & drag (outside polygon)	Move polygon
Delete	Delete single point

18 XTension

Vesicle Outside Cell

18.1 Vesicle Outside Cell

The XTVesicleOutsideCell function calculates for each Spot object the closest Cell and the distance to it.

XT VesicleOutsideCell Illustration

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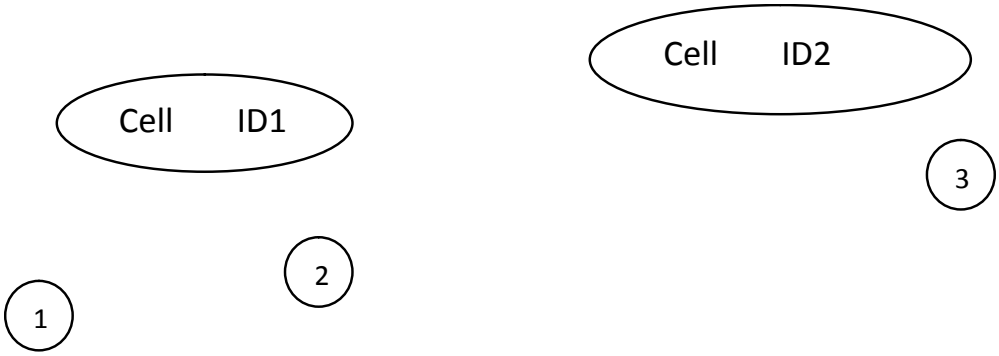

The figure illustrates two Cells (ID 1, ID 2) and three Spots (1, 2, 3).

The results od the XTVesicleOutsideCell function are two statistic values per Spot object:
Nearest Cell ID and Nearest Cell Distance.

Spot 1
Nearest Cell ID: 1
Nearest Cell Distance: 10 um

Spot 2
Nearest Cell ID: 1
Nearest Cell Distance: 7 um

Spot 3
Nearest Cell ID: 2
Nearest Cell Distance: 3 um

18.2 Similarity Statistic Values

The Similarity Statistic Values is a XT extension that allows quantifying the degree of the similarity of a set of objects with the pre-selected reference object(s).

This function creates the new statistical value, which can be used under the Filter, Statistical and Color tab.

The newly created statistical value is based upon the aggregate weighted value of the statistical characteristics of the selected object(s).
The benefits of the Similarity Statistic Values function are the precise identification of the objects with similar complex statistical properties or on the other hand, it offers the benefit of determining the objects which are the least similar to the reference object.

19 Addendum

In this addendum you find the Imaris global Mouse & Keyboard PC and Mouse & Keyboard Mac functions, tips and tricks how to customize your Imaris Interface in daily routine, and a Terminology table with frequently used technical terms and specific image processing terms.

19.1 Mouse & Keyboard PC

In the following you find the global Mouse & Keyboard functions.

Global Mouse & Keyboard Functions

Ctrl + 1	Activates the Slice view
Ctrl + 2	Activates the Section view
Ctrl + 3	Activates the Gallery view
Ctrl + 4	Activates the Easy 3D view
Ctrl + 5	Activates the Surpass view
Ctrl + 6	Activates the Key Frame Animation
Ctrl + 7	Activates the Coloc view
Ctrl + 8	Activates InMotion
Ctrl + A	Sets zoom factor to 1 pixel per voxel and centers the image to the window
Ctrl + B	Adjusts zoom factor and pan position to best fit the image into the window
Ctrl + C	Copies current view to the clipboard
Ctrl + D	Shows the Display Adjustment window
Ctrl + E	Saves Surpass scene file
Ctrl + F	Refreshes the textures of all views
Ctrl + I	Allows to inspect and edit the image properties
Ctrl + L	Opens Surpass scene file
Ctrl + N	Toggles display of Navigation window
Ctrl + O	Opens existing file
Ctrl + P	Allows to edit the settings of Imaris
Ctrl + Q	Exits the application
Ctrl + R	Discards all changes and opens the current data set again
Ctrl + S	Saves the current data set
Ctrl + T	Saves the current view as a *.tif file
Ctrl + W	Crops X, Y, or Z
Ctrl + Z	Revokes the last action
Ctrl + Shift + A	Adds channels of another image
Ctrl + Shift + B	Deletes selected channels
Ctrl + Shift + C	Copies the Imaris version, date and build to the clipboard
Ctrl + Shift + P	Allows to edit figures and annotations
F1	Opens Reference Manual
F2	Displays or updates the license status
F3	Automatic check for updates
F11	Maximizes the viewing area to the full size of your monitor
Ctrl + F1	Opens Quick Start Tutorials
Alt + F4	Exits the application
Del	Deletes selected objects
Right-click	Opens Context Sensitive Help

Global Mouse & Keyboard Functions to Navigate in All Imaris Views

Middle-click & drag	Move up: zoom out
	Move down: zoom in
Right-click & drag	Pan image
Left-click & drag	Rotate image

Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective chapter in this Reference Manual for details.

Menu Edit -	Show Display Adjustments - Mouse & Keyboard PC
Slice View -	Mouse & Keyboard PC

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Section View -	Mouse & Keyboard PC
Gallery View -	Mouse & Keyboard PC
Easy 3D View -	Mouse & Keyboard PC
Surpass View -	Overview - Mouse & Keyboard PC
	Clipping Plane - Mouse & Keyboard PC
	Contour Surface -Shortcuts Overview
	External Object - Mouse & Keyboard PC
	Filament - Mouse & Keyboard PC
	Light Source - Mouse & Keyboard PC
	Measurement Point - Mouse & Keyboard PC
	Oblique Slicer - Mouse & Keyboard PC
	Ortho Slicer - Mouse & Keyboard PC
	Surface-Shortcuts Overview
	Spots - Mouse & Keyboard PC
Animation -	Mouse & Keyboard PC
InMotion -	Mouse & Keyboard PC
Coloc -	Mouse & Keyboard PC

Context Sensitive Help

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the massage the Reference Manual opens in the actual browser and displays the corresponding page.

19.2 Mouse & Keyboard Mac

In the following you find the global Mouse & Keyboard functions.

Global Mouse & Keyboard Functions

Command + 1	Activates the Slice view
Command + 2	Activates the Section view
Command + 3	Activates the Gallery view
Command + 4	Activates the Easy 3D view
Command + 5	Activates the Surpass view
Command + 6	Activates the Key Frame Animation
Command + 7	Activates the Coloc view
Command + 8	Activates InMotion
Command + A	Sets zoom factor to 1 pixel per voxel and centers the image to the window
Command + B	Adjusts zoom factor and pan position to best fit the image into the window
Command + C	Copies current view to the clipboard
Command + D	Shows the Display Adjustment window
Command + E	Saves Surpass scene file
Command + F	Refreshes the textures of all views
Command + I	Allows to inspect and edit the image properties
Command + L	Opens Surpass scene file
Command + N	Toggles display of Navigation window
Command + O	Opens existing file
Command + ,	Allows to edit the settings of Imaris
Command + Q	Exits the application
Command + R	Discards all changes and opens the current data set again
Command + S	Saves the current data set
Command + T	Saves the current view as a *.tif file
Command + W	Crops X, Y, or Z
Command + Z	Revokes the last action

Command + Shift + A	Adds channels of another image
Command + Shift + B	Deletes selected channels
Command + Shift + C	Copies the Imaris version, date and build to the clipboard
Command + Shift + P	Allows to edit figures and annotations
F1	Opens Reference Manual
F2	Displays or updates the license status
F3	Automatic check for updates
Command + F11	Maximizes the viewing area to the full size of your monitor
Command + F1	Opens Quick Start Tutorials
Del	Deletes selected objects
Ctrl-click	Opens Context Sensitive Help

Global Mouse & Keyboard Functions to Navigate in All Imaris Views

With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image
Click & drag	Rotate image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective chapter in this Reference Manual for details.

Menu Edit -	Show Display Adjustments - Mouse & Keyboard Mac
Slice View -	Mouse & Keyboard Mac
Section View -	Mouse & Keyboard Mac
Gallery View -	Mouse & Keyboard Mac
Easy 3D View -	Mouse & Keyboard Mac
Surpass View -	Overview - Mouse & Keyboard Mac Clipping Plane - Mouse & Keyboard Mac Contour Surface - Shortcuts Overview External Object - Mouse & Keyboard Mac Filament - Mouse & Keyboard Mac Light Source - Mouse & Keyboard Mac Measurement Point - Mouse & Keyboard Mac Oblique Slicer - Mouse & Keyboard Mac Ortho Slicer - Mouse & Keyboard Mac

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	Surface-Shortcuts Overview
	Spots - Mouse & Keyboard Mac
Animation -	Mouse & Keyboard Mac
InMotion -	Mouse & Keyboard Mac
Coloc -	Mouse & Keyboard Mac

Context Sensitive Help

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the massage the Reference Manual opens in the actual browser and displays the corresponding page.

19.3 Customize Interface

In this chapter you find a collection of tips and tricks to customize the Imaris interface for your daily routine.

Histogram

Logarithmic or linear histogram display.

- On the left hand side of a histogram there are horizontal lines representing a linear or logarithmic histogram display.
- Click on these lines to change the display from logarithmic to linear and vice versa.

The default setting is logarithmic.

Windows

You can plug windows in Imaris in or out.

Example: Key Frame Animation window

Plug out:
- Click on the header of the Key Frame Animation window and drag the window to any free space on the screen.
- On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged out, you can drag it on any free space on the screen.

Plug in:
- On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged in on its original place.

Tabbed Toolbars

Try to undock the toolbars and dock them all at the same side. Especially in combination with InPress, this saves a lot of screen area.

Example: In the Section view you can bring the toolbar Figures (InPress), Camera, and Properties to the left side at the bottom.

Objects Toolbar

You can adjust the Objects toolbar to your personal preferences.

- Open the menu Edit - Preferences - Surpass - Object Creation Buttons to adjust your personal Objects toolbar.

Display Mode Blend and Bland Opacity

You can select the Display Mode Blend in the Surpass view for a Volume object, in the Section view (Extended) and in the Easy 3D view. Any change in the Display Adjustment window in the parameter Blend Opacity is only visible, if a Blend Mode is selected.

19.4 Terminology

Term	Description
Channel	A channel is a particular piece of information that has been recorded for all voxels. Most of the time in light microscopy, the different channels are intensities that have been measured at different wavelengths. For instance, a two-channel data set contains two intensities for each voxel, one intensity measured in channel 1 (e.g., green light) and one intensity in channel 2 (e.g., red light). Ideally, the intensity values present in different channels are independent of one another.
Coordinates	Any position in the data set can be described either in the voxel grid (e.g., voxel no. 5 in x-direction) or by a coordinate, which corresponds to a m). The distance relative to a defined origin in the real world (e.g. 2 latter) requires the correct calibration of the data set, i.e. that the voxel size is defined appropriately. Generally, in Imaris the origin of the data set is in the left bottom corner. If the data set was cropped from a larger data set, the bottom left corner may have minimum coordinates that are not equal to (0,0,0).
Image stack	A 3D data set is sometimes called an image stack consisting of 2D images stacked on each other.
MIP	A maximum intensity projection (MIP) is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.
Original Data Set	A 3D data set that cannot be derived from any other data set using Bitplane software. Usually "Original data sets" have been acquired using an image sensor and are loaded into Imaris.
Rendering	A technique that visualizes a multi-dimensional data set as a two-dimensional image that can be displayed on a computer screen or printed on a laser printer.
Resultant Image	An image that has been computed using Imaris.
Standard Deviation	In probability and statistics, the standard deviation of a probability distribution, random variable, or population or multiset of values is a measure of the spread of its values. It is defined as the square root of the variance. The standard deviation is the root mean square (RMS) deviation of values from their arithmetic mean. For example, in the population {4, 8}, the mean is 6 and the standard deviation is 2. This may be written: {4, 8} 6. In this case 100% of the values in the population are at one standard deviation of the mean.

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	The standard deviation is the most common measure of statistical dispersion, measuring how widely spread the values in a data set are. If the data points are close to the mean, then the standard deviation is small. Conversely, if many data points are far from the mean, then the standard deviation is large. If all the data values are equal, then the standard deviation is zero. The standard deviation (s) of a population can be estimated by a modified standard deviation (s) of a sample.
Threshold	A threshold in Imaris is a gray value, which acts as a limit. All gray values above (or below) are assigned a particular functionality.
Time Point	A time point is a single 3D image containing one or many channels. The term is used regardless of what data is actually stored in the channels. In particular, the channels could contain data taken at different points in time with a changing object.
Volume Rendering	A rendering technique which operates directly on the voxel data. The input image consisting of a collection of voxels arranged in a regular grid is converted directly into the 2D output image.
Voxels	A volume data set is composed of volume elements called voxels. The voxels are the smallest units within the image about which we have distinct information in the form of a measured intensity. The dimensions of an individual voxel are specified in most image file formats supported by Imaris.